CHANGES IN ANTHROPOMETRIC AND METABOLIC OUTCOMES DURING AND AFTER A MILK-BASED MEAL REPLACEMENT PROGRAMME IN BARIATRIC PATIENTS.

A Thesis Submitted By

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For The Degree Of

DOCTOR IN MEDICINE (M.D.)

At

National University of Ireland Galway

November 2019

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Declaration

I hereby declare that this thesis entitled "CHANGES IN ANTHROPOMETRIC AND METABOLIC OUTCOMES DURING AND AFTER A MILK-BASED MEAL REPLACEMENT PROGRAMME IN BARIATRIC PATIENTS.", which I submit to National University of Ireland, Galway for the examination in consideration of award a higher degree of Doctor in Medicine is my own personal effort with support and help of my supervisor professor Francis Finucane. I have acknowledged all main sources of help. I have not already obtained a degree in National University of Ireland, Galway or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and to the best of my knowledge, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

RAZK H. ALI ABDALGWAD

November 2019

Acknowledgement

First and foremost, praises and thanks to the God, the Almighty, for his showers of blessing throughout my research work to complete the research successfully.

I would like to express the kindest and deepest thanks and appreciation to my supervisor, Professor Francis Finucane, who has the attitude and substance of a genius, wisdom and patience. He continually and convincingly conveyed a spirit of motivation and support regarding to research and an excitement regarding to teaching. Without his guidance and persistence help and encouragement this work would not have been possible.

I also would like to extend my sincere thanks and gratitude to Dr.Micheál Newell (Co-Supervisor), Prof. Derek O'Keeffe (research committee member), Dr.Colin Davenport (endocrine specialist), for their support, help, and guidance in my research project.

I also would like to extend my thanks to my friend Dr. Faraz Rafey, for helping me in my research, collecting, and analyzing of data.

I profusely thank our team, all nurses in our bariatric clinic including Helena Griffin, and Siobhan Foy, and the dietitian, for their co-operation and the great time that we had together. I would also like to thank Thomas J Mellett and the nursing staff in the phlebotomy department UHG, for teaching me.

I must extend my thanks to biochemistry team for teaching and guidance including Dr.Paula O'Shea and Dr.Liam Blake.

Thanks should also go to our patients who were kind to give me the opportunity and their cooperation to get involved in my research study.

I dedicate this work to my father Hamad, mother Fadilah, and my brothers and my sisters and my friends Esmael Hamuda and Ramadan Ahmed for supporting me spiritually throughout writing this project and my life in general.

Finally, I would like to thank Ministry of Higher Education Libya for the fund and scholarship, and National University of Ireland, Galway, Ireland for providing good facilities and atmosphere to support my research study.

Summary

During this work, I examined a cohort of patients with severe and complicated obesity undergoing a milk-based intensive weight management programme, examining changes in various traits in the short and medium term.

Firstly, I helped to conduct a retrospective cohort study to examine the effect of this programme on weight and other anthropometric and metabolic variables among these patients over 24 weeks. The results of this study showed significant improvements in weight, BMI, HbA1c, lipid profiles and a reduction in most prescribed medications. Blood pressure did not significantly change, but there was a reduction in antihypertensive medication use.

Thereafter, I described changes in alanine aminotransferase (ALT) levels over 24 weeks of the milk diet and unexpectedly I found that in the first eight weeks of the intervention, there was an increase in ALT and it was more pronounced in the group with high baseline ALT than in those with normal baseline ALT. I noted a significant reduction in ALT at the end of the programme which might indicate a reduction in liver fat content and steatosis.

Finally, a more prolonged retrospective longitudinal cohort study was conducted by me to investigate the long-term changes in weight in completers of the milk diet. I showed that after initial significant weight loss there was regain in weight, most apparent and significant in the third and fourth years of follow up but this regain did not exceed the weight at the beginning of the programme. I noted that at six months, the degree of weight loss predicted weight regain in the first and third years of follow up (inverse relationship).

List of Abbreviations

ACEIs	Angiotensin Converting Enzyme Inhibitors
ACG	American College of Gastroenterology
AgRP	Agouti-related peptide
ALT	Alanine Aminotransferase
ANOVA	Analysis of variance
ARBs	Angiotensin Receptor Blockers
AST	Aspartate Aminotransferase
BBs	Beta Blockers
BMI	Body Mass Index
CART	Cocaine- and Amphetamine-Regulated Transcript
CCK	Cholecystokinin
CHF	Congestive Heart Failure
CI	Confidence Interval
CVD	Cardio vascular Disease
DASH	Dietary Approaches to Stop Hypertension
DBP	Diastolic Blood Pressure
DPP4	Dipeptidyl Peptidase 4 Inhibitor
DM	Diabetes Mellitus
EBW%	Percentage Excess Body Weight
EWL%	Excess Weight Loss Percentage
FTO	Fat Mass-and Obesity Associated Gene
GDPR	General Data Protection Regulation

GIP	Gastric inhibitory polypeptide
GLP 1	Glucagon –Like Peptide 1
GPO-PAP	Glycerol-3-Phosphate Oxidase-Phenol + Aminophenazone
GUH	Galway University Hospital
HbA1c	Glycated Hemoglobin
HBDs	Hypoenergetic Balanced Diets
HDL	High Density Lipoprotein
HOMA	Homeostatic Model Assessment
HPLC	High-performance Liquid Chromatography
HR	Hazard Ratio
IHF	Intrahepatic Fat
IQR	Interquartile Range
KJ	Kilojoules
Kg	Kilogram
LAGB	Laparoscopic Adjustable Gastric Banding
MC4R	Melanocortin-4-Receptor gene
MJ	Milli-Joules
MRI	Magnetic Resonance Imaging
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
NEFA	Non-Esterified Fatty Acid
NICE	National Institute for Health and Care Excellence
NPY	Neuropeptide Y

NREE	Non-Resting Energy Expenditure
OAGB	One-Anastomosis Gastric Bypass
OLGB	Omega-Loop Gastric Bypass
PAF	Population Attributable Fraction
PCOS	Poly Cystic Ovary Syndrome
POMC	Pro-opiomelanocortin
PPARG	Peroxisome proliferator-Activated Receptor Gamma
PP	Pancreatic Polypeptide
PYY	Peptide YY
RCTs	Randomized Controlled Trials
REE	Resting Energy Expenditure
RR	Relative Risk
RYGB	Roux-en-Y Gastric Bypass
SBP	Systolic Blood Pressure
SG	Sleeve Gastrectomy
SGLT2	Sodium-glucose co-transporter-2 Inhibitor
SNP's	single nucleotide polymorphisms
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
SU	Sulfonylureas
TCA	Tricarboxylic Acid Cycle
T2DM	Type 2 Diabetes Mellitus
TWL%	Percentage Total Weight Loss
VLCD	Very Low Calorie Diet

VLEDs	Very-Low Energy Diets

List of Figures

List of Tables

Publications and Presentations Arising from the Thesis

1. **"Effects of a milk-based meal replacement program on weight and metabolic characteristics in adults with severe obesity."**

   Mohammed F. Rafey, Conor F. Murphy, **Razk Abdalgwad**, Katriona Kilkelly, Helena Griffin, Niamh Beaty, Paula M O'Shea, Chris Collins, Robert McGrath, Mary Hynes, Colin Davenport, Martin O'Donnell, Francis M Finucane.

   Published at "Diabetes, Metabolic Syndrome and Obesity: Target and Therapy."

2. **"Changes in Alanine Aminotransferase in Adults with Severe and Complicated Obesity during a Milk-Based Meal Replacement Programme."**

   Razk Abdalgwad, Mohammed F. Rafey, Conor Murphy, P.M. O'Shea, Eoin Slattery, Colin Davenport, Derek .T O'Keeffe, Francis M. Finucane.

   Poster presentation at Irish Endocrine Society 2019.

   Under review at "Annals of Clinical Biochemistry."

3. **"Long-term Changes in Weight in Patients with Severe and Complicated Obesity after Completion of a Milk-Based Meal Replacement Programme."**

   Razk Abdalgwad, Mohammed F Rafey, Siobhan Foy, Colin Davenport, Derek T O'Keeffe, Francis M Finucane.

   Abstract accepted as a poster presentation at ECO-ICO 17-18 May 2020.

   Submitted to "Frontiers in Nutrition."

Table of Contents

Chapter four: Long-term Changes in Weight in Patients with severe and complicated Obesity after completion of a Milk-based diet programme.

Chapter five: Conclusion

Chapter One:

Introduction.

1.1: Background:

Obesity is a highly prevalent, complex disease associated with increased morbidity and mortality. [1-3] Obesity can contribute to the development of many serious diseases that can affect one's health and life expectancy such as diabetes, cardiovascular disease, hypertension, NAFLD, and certain types of cancers. [4, 5] Therefore, management of this condition has become a concern for patients and clinicians. [6] Non-surgical treatment approaches to weight loss include; dietary interventional management programmes, exercise, and medications. Losing excess body weight can be achieved, but weight regain is challenging. [7] According to the most recent evidence surgery is still superior to other interventions in terms of long-term weight loss [8], although as with any surgical procedures it still carries risks of complications. [9] The objective of this current set of studies is to assess in short-term and long-term the effect of a milk diet on weight in particular, and other anthropometrc and metabolic outcomes. Also, this study will explore the effect of the milk diet in the shorter term on alanine aminotransferase (ALT) (an indicator of liver injury) in patients with severe and complicated obesity.

1.2: Prevalence of obesity:

Worldwide, obesity is considered as a significant health problem, causing devastating morbidity and mortality.[1, 2] Recent epidemiological studies have indicated that the prevalence of obesity is noticeably increasing.[10] Globally, from 1980 to 2013, the prevalence of obesity and overweight increased by 27.5%, and 47.1% for adults, and children, respectively.[11] In the United States, a survey was conducted to analyze the change in the trends of obesity among children and adult individuals over a ten-year period starting from 2003 through to 2012; the prevalence of obesity has not significantly changed over that period, but remains considerably high particularly among women aged 60-years and older (from 31.5%to 38.1%; P = 0.006).[12] In Canada, the prevalence of obesity is less than that of the United States reaching 27% and 25% of Canadian males and females, in turn.[13] In Europe, the prevalence of obesity ranges from 4% to 28.3% for men and from 6.2% to 36.5 % for women. The prevalence rates were lower in Western and Northern Europe than the other parts of Europe.[14] More than 50% of European individuals are overweight and obese.[15] Obesity also increased in adults with T2DM fluctuating from 50.9% to 98.6 %.[16, 17]

A study carried out in Argentina showed that obesity in years 2008 to 2014 increased from 21.3% to 26.9% among males, and from 15.2% to 23% among females, the highest prevalence of obesity was observed for men aged 40-49 years and women aged 50-59 years.[18] In Spain, the prevalence of obesity is 24.4% in males, and 21.4% in females, respectively. Moreover, 32%, and 39% of men and women had abdominal obesity, in turn.[19] A study was carried out in Ireland to determine the prevalence of overweight and obesity among primary school children which indicated that from 2002 to 2012 the obesity prevalence rates is still high and seems to be plateaued at that higher level. Additionally, about 25% of Irish children are either overweight or obese.[20] A survey was conducted in Northern and Republic of Ireland from 1997 to 1999 which showed that weight and BMI increased significantly during that period.[21] Overall, it seems that the prevalence of obesity is still increasing in different countries around the world.

1.3: Mechanisms and aetiology of obesity:

Obesity is multifactorial in origin and many independent risk factors such as imbalance in energy intake and energy expenditure [22], lack of physical activity, genetic factors, behavioural, and environmental factors are all contribute in the development of obesity.[23]

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Figure 1.3.1 Mechanisms involved in body weight regulation. Figure reproduced from [24]

NPY = neuropeptide Y, AgRP = agouti-related peptide, GIP = gastric inhibitory polypeptide, POMC = pro-opiomelanocortin , CART = cocaine- and amphetamine-regulated transcript , GLP 1 = glucagon likepeptide 1 , PP = pancreatic polypeptide, CCK = cholecystokinin , PYY = peptide YY, REE = resting energy expenditure, NREE = non-resting energy expenditure.

Under steady-state condition normal body weight is controlled by three main factors namely homeostatic, environmental, and behavioural (Figure 1.3.1).[24] Any imbalance between these factors could contribute to weight gain. g Haemostatic factors, namely

the hypothalamus, the caudal brainstem and parts of the cortex and limbic system respectively [25, 26], play an important role in the regulation of body weight, food intake, and energy balance, by responding to several hormones and neuropeptides that control appetite regulation. Some of these hormones and neuropeptide act centrally in the hypothalamus to regulate appetite, some of them are hunger stimulator such as neuropeptide Y(NPY), agouti-related peptide (AgRP), while others suppress hunger such as cocaine- and amphetamine-regulated transcript (CART), and pro-opiomelanocortin (POMC).[25, 27] Additionally, the hypothalamus responds to peripheral signals that transform information about short-term food intake (e.g. nutrient availability), or long-term energy balance (e.g. energy stores) to maintain energy homoeostasis.[28, 29] Short-term signals include hunger-stimulating hormones such as ghrelin and gastric inhibitory polypeptide (GIP), while hunger-suppressing hormones are glucagon-like peptide 1 (GLP 1, peptide YY (PYY), and cholecystokinin (CCK) that are secreted from the gastrointestinal tract; the hunger-suppressing hormones from the pancreas are pancreatic polypeptide (pp), amylin, and insulin. Although insulin can cause weight gain when used peripherally to treat diabetes, and the hunger-suppressing hormone from adipocytes is leptin.[25, 26] The hypothalamus is also responsible for integrating signals from 'hedonic reward pathways in the cortico-limbic system that are associated with the pleasure drive for eating (for example, sight, smell, and taste).[25]

Another contributing factor for obesity is the environment that we live in. This environment can have a negative impact in many ways such as intense marketing of energy-dense foods, large portion sizes, and a high-stress society that contribute to compensatory high food intake.[30] Finally, specific changes in behaviours such as eating a healthy diet and regular exercise can contribute to the maintenance of weight loss.[31]

Imbalance between energy intake and energy expenditure:

Not only has the imbalance between energy intake and expenditure been linked to weight gain, but also the nature and the composition of consumed food. For example food that is rich in carbohydrates such as pasta, potatoes, rice, and bread is related to the development of obesity. Actually, they can increase blood sugar and insulin secretion themselves greater than high sugar content food.[32, 33] Hyperglycemia and hyperinsulinaemia are risk factors for the development of type 2 diabetes, coronary

artery disease and obesity.[34] Insulin stimulates glucose uptake into cells and inhibits fatty acids release from adipose tissue, preventing hepatic ketone synthesis and stimulating glycogen and fat deposition.[35]

Reduced physical activity:

Indeed a lack of physical activity is a risk factor for obesity and overweight and their related health consequences.[36] In one study, among adolescents physical inactivity was associated with an odds ratio of (3.9, 95% CI 1.4-10.9) of increased risk of obesity, and 4.8 (1.9-12.0) increase risk of abdominal obesity at 25 years old.[37] Another study in Ireland showed that the prevalence ratio of physical inactivity among Irish Travellers with cardiovascular disease was 1.3, (95% CI 1.2 to 1.4) which is higher than those without cardiovascular disaese.[38] In Ireland a longitudinal child cohort study showed that children with chronic illness were more likely to be either obese or overweight. Moreover, children with chronic illness tended to be less physically active.[39]

In Sri Lankan adults, physical inactivity was significantly associated with overweight and obesity [odds ratio (95% CI): 1.4 (1.1 to 1.7), $p = 0.007$].[40] A systematic review of school-aged youth from 34 countries showed that the prevalence of overweight and obesity was higher in countries of North America, UK, and south-western Europe, and majority of countries had lower levels of physical activity.[41] In a multi-centre study conducted in Spain, physical activity, sedentary lifestyles, and levels of adiposity were strongly associated in individuals of 65 year old or more.[42]

Genetic factors:

Several genes like Melanocortin-4-Receptor gene (MC4R) and Fat Mass-and Obesity associated gene (FTO) were found to be strongly associated with obesity-related traits.[43, 44] the rs1943218 minor allele is referring to the MC4R gene was associated with short- (from baseline to six months) and long-term (from baseline to two years) weight loss. Additionally, 7 more single nucleotide polymorphisms (SNPs) were found to be associated with short-term (rs17066856, rs9966412, rs17066859, rs8091237, rs17066866, rs7240064) or long-term (rs12970134, rs17066866) weight loss. Of these rs7240064 in the placebo group and rs9966412 and rs17066859 in the metformin group were associated with significant weight change.[43] The FTO gene was found to be associated with variation in energy intake and possibly to affect on obesity through food

intake instead of energy expenditure.[44] Studies of mouse Fat Mass-and Obesity associated gene (FTO) and human Fat Mass-and Obesity associated gene messenger Ribonucleic Acid (FTO mRNA) expression have shown that mouse FTO and human FTO mRNA are widely distributed in many tissues, but the highest expression was found in the brain, particularly the arcuate nucleus of the hypothalamus. [45, 46] Other studies of FTO mRNA expression in wild-type rodent tissues showed that the highest levels of FTO mRNA were found in hypothalamic regions which are responsible for the regulation of energy intake and expenditure and suggested that expression of FTO might be regulated by nutritional status.[47, 48] During fasting state a significant reduction in expression of hypothalamic FTO mRNA was observed in obese mice as compared to fed control. [47, 48] Moreover, polymorphisms in the FTO gene were found to be strongly associated with BMI and obesity among a Chinese population with a modest effect.[49]

Among Chilean adults uncontrolled eating scores in women but not men were statistically significantly associated with BMI (P = 0.002), and uncontrolled eating scores were found to be higher in C-allele carriers of MC4R-rs17782313 than non carriers.[50] In another study, carriers of the G allele who underwent behavioural weight reduction programmes based on the Mediterranean diet did not lose more weight as compared to non-carriers, and mutation in the rs1801260 CLOCK gene was associated with obesity at baseline.[51]

Several single gene defects in leptin melanocortin pathway implicated in the aetiology of severe obesity have been identified.[52] For example, in 1997, two children of Pakistani origin with severe obesity were found to have the defect in the *LEP* gene (ΔG133); both children had low levels of serum leptin.[52, 53] Clinically, phenotypes related to congenital leptin and leptin receptor deficiencies are not different. In individuals with deficiency in leptin and leptin receptor birth weights are normal. Though, fast weight gain in the first few months of life among these patients leading to severe obesity was observed.[54] Leptin deficiency is associated with excess subcutaneous fat deposition in the trunk and limbs.[54] Administration of recombinant human leptin to these children has resulted in dramatic reductions in weight, and fat mass.[54, 55] Leptin 25CAG allele was found to be high in 53 obese Japanese patients.[56]

1.4: Comorbidities associated with obesity:

It is well known that obesity is a risk factor for the development of many diseases such as diabetes, hypertension, cardiovascular disease, NAFLD, NASH, and certain types of cancer.[4, 5]

In a survey conducted in Ireland, there was a significant correlation between obesity and pre-diabetes. Population attributable fraction (PAF) as an estimate for pre-diabetes was 31.3% (95% CI -3.9 to 54), 10.0% (95% CI -2.7 to 21.3), and 6.1% (95% CI -4.9 to 15.9) for excess BMI, physical inactivity, and poor diet, respectively.[57] In another study, the prevalence of diabetes was higher in obese patients at 43% as compared to those with normal weight at only 8%.[58] In Australia, among obese individuals the prevalence of diabetes was 37%.[59] In Canada, the prevalence of obesity associated with diabetes was 44% for men and 53% for women.[60]

Obesity even in metabolically healthy individuals is associated with a risk of coronary artery calcification and atherosclerosis.[61] In another study, obesity was associated with increased risk of death from cardiovascular disease (CVD) with hazard ratio (HR) of 1.86 [95% CI: 1.50 to 2.31].[62] Similarly, obesity was associated with a significant increase in CVD mortality.[62] In another study, BMI of ≥ 35 kg/m^{-2} was associated with relative risk (RR) of 2.13 [95% CI: 1.82 to 2.48], and 2.48 [95% CI: 2.20 to 2.80] increased risk of congestive heart failure (CHF), in men and women, respectively.[63]

NAFLD and NASH are highly prevalent among obese individuals.[64] In severely obese patients who underwent bariatric surgery, the prevalence of NASH was 9.8%, and BMI among these patients was the only independent predictive factor for NASH.[65] In a prospective study of obese patients who underwent gastric bypass surgery, NAFLD was found in 63% of patients, and steatosis was diagnosed in 37%, and 33% of patients had NASH.[66] In another study assessing the prevalence of NASH and NAFLD among patients with morbid obesity found that the prevalence of NAFLD was in 93% of patients and of these 26% had NASH.[67]

In many studies, certain types of cancer have been found to be associated with obesity and high BMI such as esophageal cancers, breast in postmenopausal women, endometrial cancers, colorectal, pancreas, thyroid, liver, kidney, and gallbladder.[68-77] In a meta-analysis, obesity was associated with increased risk of 1.19 [95% CI, 1.11 to 1.29] of colorectal cancer, and those with highest central obesity there was an increased risk

of 1.45 [95% CI, 1.31 to 1.61] of colorectal cancer.[78] In another meta-analysis, overweight and obese patients were found to have increased risk of developing gallbladder cancer at 1.15 [95% CI, 1.01 to 1.30], and 1.66 [95% CI, 1.47 to 1.88], respectively.[79] In Austria, a large cohort study examining the association between overweight and obesity in 145000 adults found that increased BMI of ≥ 35 kg/m^{-2} in men was associated with increase in risk of colon cancer at [hazard ratio (HR) 2.48; 95% CI: 1.15 to 5.39], and pancreatic cancer at HR 2.34; 95% CI: 1.17 to 4.66 for those with BMI of > 30 kg/m^{-2}].[80]

1.5: Interventions used to treat overweight and obesity:

Weight loss among obese individuals has been associated with improvements of many obesity-related health conditions such as hypertension, cardiovascular diseases, diabetes, NAFLD, and sleep apnea. [81] Even modest weight loss of 5% to less than 10% was associated with improved health.[82] Though, some argued that 15% loss of total body weight especially in severely obese patients is necessary to obtain more health benefit. [83] Weight loss can be achieved surgically through bariatric surgeries, and non – surgically by using diet, and lifestyle programmes, exercise, and medications. In a study aimed to determine the response to low-fat and low carbohydrate diets in two groups of patients (more insulin resistance group, and more insulin sensitive group), at 6 months weight loss was 7.4 ± 6.0, and 10.4 ± 7.8 kg in low-fat insulin resistance and low-fat insulin sensitive groups, respectively, while it was 9.6 ± 6.6, and 8.6 ± 5.6 kg among low-carbohydrate insulin resistance and low-carbohydrate insulin sensitive groups, in turn.[84] In another meta-analysis examining long-term weight loss maintenance (for ≥ 2 years) between completers of very-low energy diets (VLEDs) of < 800 Kcal/day (3347 Kilojoules), and hypoenergetic balanced diets (HBDs) which approximately equal to 1000 Kcal/day (4180 Kilojoules) showed that the group of VLEDs maintained more weight loss than the group of HBDs at 7.1 kg (95% CI: 6.1, 8.1), and 2 kg (95% CI: 1.5, 2.5).[85] An eight week supervised structured lifestyle programme showed a significant reduction in weight and improvement in other anthropometric and metabolic traits including adiposity, fitness, diabetes, and cardiovascular risk factors in 150 patients with severe and complicated obesity [with body mass index (BMI) of ≥40 kg/m^{-2} (or ≥35 kg/m^{-2} with significant co-morbidity)].[86]

In a meta-analysis aimed to assess different types of non-surgical weight loss interventions, those that used low energy diet and/or weight loss medications such as orlistat, and sibutramine all resulted in 5% to 9% loss of total body weight at 6 months, with weight levelling off at approximately 6 months. However, the studies that had duration of 2 years the percentages of weight loss ranged from 3% to 6%, with no reported weight regain from the baseline. There was only slight weight loss among groups of advice only or exercise alone.[87] Additionally, a meta-analysis reported that diet, and exercise combination programme associated with more weight loss maintenance than a diet-only programme. Though, both programmes were associated with slight weight regain.[88] In another meta-analysis that was conducted to examine the effect of long-term lifestyle programmes on weight loss and cardiovascular risk factor among obese and overweight patients showed that diet and exercise combined together resulted in more significant weight loss than diet alone and exercise alone. When comparing diet to exercise, diet was found to have a significant effect on weight loss.[89]

In addition to above mentioned interventions to lose weight, several medications have been made available to treat obesity (table 1.5.1).[90, 91] For example, liraglutide, a glucagon –like peptide 1 (GLP 1) receptor agonist can be used for the purpose of weight loss. It acts by stimulating insulin secretion, inhibiting glucagon secretion, decreasing gastric emptying and increasing satiety after eating. It is labeled as an adjunct to dietary and exercise interventions to treat obesity. The starting dose of this drug is 0.6 mg once a day subcutaneous injection, increasing by 0.6 mg weekly to target a maximum dose of 3 mg once a day) used to treat obesity, while for T2DM according to National Institute for Health and Care Excellence (NICE) guideline, which was published 2010, recommended the use of liraglutide at dose of 1.2 mg/day in triple therapy regimens combined with metformin and sulfonyl urea, or metformin and thiazolidinedione for treatment of patients with T2DM when control of blood glucose remain high at HbA1c of ≥ 7.5%, and to fulfill the criteria specified by NICE patients must have body mass index (BMI) ≥ 35 kg/m^{-2} in those of European descent and specific psychological or medical problems associated with obesity.[92, 93] Also, these regimens can be used for those with BMI ≤ 35 kg/m^{-2} if the treatment with insulin would cause significant occupational implications or if weight loss would improve other significant obesity related complications. The higher dose of liraglutide (1.8 mg/day) is not recommended by NICE to treat T2DM.[93] Liraglutide has been reported to be superior to orlisat in terms of significant weight loss when used for 1 and 2 years, respectively.[94] In an RCT examining

the effects of 3.0 mg liraglutide on weight loss, it is reported that more than 50% of participants achieved a weight loss of 5% over one year, and 10% (about 10kg) in 25% to 33% patients in the same mentioned period. Maintenance of weight loss for up to 2 years has been shown with continuous liraglutide therapy. [95] However, weight regain may occur when the drug discontinued. [94] Reduction of weight to about 5%-10% of total body weight for obese patients has been proved to be sufficient to reduce the risk of stroke and cardiovascular diseases.[27] In one study, once weekly semaglutide resulted in a mean body weight loss of 5.0 kg.[96] In a randomized control trial phentermine/topiramate resulted in 5% body weight loss with improvements in metabolic outcomes.[97] Similarly, naltrexone/bupropion has been shown in one trial to achieve a body weight loss of ≥ 5% among treated participants at week 56 more than control at 50.5%, and 17.1%, respectively.[98] Moreover, in a multicenter, placebo-controlled trial, lorcaserin when combined with behavioural modification was associated with dramatic weight loss of 10.9% and resulted in weight loss maintenance more than placebo.[99] A four year double-blind, randomized trial reported mean weight loss of 5.8 kg for orlistat group compared to 3.0 kg in placebo group; $p < 0.001$.[100]

Bariatric surgery is the only intervention that has the most significant and successful long-term weight loss compared to the other weight loss interventions.[8] In a review of the key results from The Swedish Obese Subjects trial, authors noticed that bariatric surgery was the only management for obesity resulting in > 15% of weight loss over 10 year period, and this was concomitant with improvement in cardiometabolic risk factors. For example, diabetes preventive effect of bariatric surgery was specifically dramatic in patients with impaired glucose tolerance. Overall mortality of cardiovascular disease and cancer were reduced after bariatric surgery.[8] Moreover a systematic review and meta-analysis showed that EWL% in the studies of gastric bypass surgeries was 56.7%, while the studies of biliopancreatic bypass with or without duodenal switch resulted in about 74.1 EWL%. Participants who had sleeve gastrectomy lost 58.3% of EWL. Though, reoperations were commonly seen among all groups, at twenty years, weight loss was 30.1 kg, EWL% was 48.9, and TWL% was 22.2%.[101]

Table 1.5.1 Mechanism of action and common side effects of FDA approved drugs for weight loss.[90, 91, 102]

Drugs (trade name)	Mechanism of action	Main side effects
Orlistat (Xenical, Alli)	GI and pancreatic lipase inhibitors; decrease lipid absorption.	Oily stools, oily spotting, fecal urgency, fecal incontinence, hyper-defecation, flatus with discharge, and deficiency of fat soluble vitamins (A, D, E, K). **Serious side effects**: Thrombocytopenic purpra, anal abscess, pain hypochondrium, liver disorders, and hypoglycemia.
Lorcaserin (Belviq, Belviq XR)	5HT-2C R agonist; decrease food intake.	Headache, dizziness, Fatigue, nausea, constipation, and dry mouth. **Serious side effects**: Acute anxiety, syncope, valvulopathy, cardiac disorder, and cancer.

Table 1.5.1 Mechanism of action and common side effects of FDA approved drugs for weight loss *(Continued).*

Liraglutide (Saxenda)	GLP 1 agonist; decrease gastric emptying, increase satiety, and decrease food intake.	Nausea, diarrhea, constipation, vomiting, and dyspepsia. **Serious side effects**: Pancreatitis and cholelithiasis.
Phentermine/Topiramate (Qsymia)	NE agonist/GABA agonist, glutamate antagonist; suppress appetite.	Paresthesia, dry mouth, constipation, insomnia, dysgeusia, anxiety, and depression. **Serious side effects**: Myelogenous leukemia, severe acidosis, renal stone, hypoglycemia, acute pancreatitis, and atrial fibrillation.
Naltrexone/Bupropion (Contrave)	Opioid receptors antagonist/DA and NE reuptake inhibitor; increase satiety, and suppress appetite.	Nausea, headache, constipation, dizziness, vomiting, and dry mouth. **Serious side effects**: MI, heart failure, seizure, suicidal ideation.

FDA = Food and Drug Administration, GI = gastrointestinal, 5HT-2C R = 5hydroxytryptamine 2C receptor; NE =norepinephrine , GABA = gamma-aminobutyric acid, GLP 1 = glucagon-like peptide 1 , DA = dopamine , MI = myocardial infarction, Dysgeusia= distortion of sense of taste.

1.6: Aims and objectives:

1. To assess in the short-term (6 months) the effects of a milk-based intensive weight management programme on weight and other anthropometric and metabolic variables in patients with severe and complicated obesity.

2. To determine the short-term (6 months) changes in alanine aminotransferase, a biomarker of liver injury, in patients with severe and complicated obesity who completed 24 weeks of a milk-based intensive weight management programme.

3. To determine the long-term weight regain among completers of a milk-based intensive weight management programme.

Chapter Two:

Effects of a milk-based meal replacement programme on weight and metabolic characteristics in adults with severe obesity.

2.1: Background:

Due to the higher prevalence of severe obesity [103], the availability of effective interventions for affected patients is increasingly required. Undoubtedly, bariatric surgery is an efficacious and cost effective intervention. [104] However, only 1 out of 10 of patients with obesity who meet the criteria for bariatric surgery agree to have this option. [105] Other branches of bariatric care need to be improved and assessed. The benefits of structured lifestyle interventions have been established in many studies among different patient subgroups, including those with comorbidities such as non-diabetic hyperglycaemia [106], prevalent cardiovascular disease [107] or established type 2 diabetes. [108, 109] However, meaningful, long-term weight loss is difficult to be maintained with lifestyle modification alone. [110] Recently, a large general practice-based cohort study of adults with severe obesity in the UK found that the proportion achieving 5% weight loss after one year was just about 12.5% of men and 14.3% for women. [111] In other words, most patients with severe obesity in the community attending primary care based services do not manage to achieve even moderate weight loss. For some, it is believed that a significant improvement in health requires a weight loss of 10%. [109] However, the improvements in health in bariatric patients were observed after more moderate weight loss of 2.7%.[112]

The use of low energy liquid diets (LELDs, ~1200 kcal/ day) as part of intensive lifestyle modification programmes for the treatment of obesity have been described in many studies. The ideal weight loss is approximately 10kg. [108, 113] Though, weight regain limits the longer term efficacy of these interventions. [110, 114] Side effects of low energy diets are constipation, dizziness, alopecia, nausea, headache, diarrhoea, abdominal pain and cholelithiasis.[115] These side effects are related to a reduction in fibre consumption, the diet itself, medications, inadequate fluid intake, or long intervals between meal replacements. [115] Implementing meal replacement interventional studies is challenging. Some of the main problems highlighted in the literature include maintaining sufficient trial numbers [108, 113], and combating attrition rates; as attrition rates can be as high as 50% .[110, 116] Another design limitation is the cost of the intervention. Commercial meal replacement programmes can be expensive, with some analyses suggesting they are prohibitively cost ineffective. [117] Semi-skimmed milk is a potential low-cost alternative to commercially produced meal replacement supplements. Milk whey protein

attenuates muscle loss [118] and preserves myofibrillar protein synthesis [119] in adults with obesity during very low calorie diets. Milk reduces appetite, calorie intake and body weight [120] and alters post-prandial glucose and lipid metabolism [121] in men with obesity. In mice, milk casein-derived peptides reduce high-fat diet-induced adipose tissue inflammation. [122] A recent trial showed that drinking low fat milk made children feel fuller and eat less later in the day compared to juice or water. [123]

There is limited data regarding the feasibility, efficacy and safety of a semi-skimmed milk-based meal replacement programme for adults with severe obesity. In the bariatric clinic of University Hospital Galway, we introduced a milk-based low energy liquid diet (LELD) in 2013, providing patients with approximately 1200 kcal/day over eight weeks, with a subsequent 16-week period of food reintroduction, as described below. The aim of this study was to conduct a retrospective analysis of patient characteristics, key anthropometric and metabolic outcomes and attrition rates in patients attending the programme, in order to inform more robust design of prospective studies or potential randomised controlled trials for future evaluations of the efficacy and safety of a semi-skimmed milk-based meal replacement programme.

2.2: Methods:

2.2.1: Study design, population and setting:

This was a single-center, retrospective cohort study, conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for Observational study. [124] The study population included bariatric patients who were referred to our milk-based meal replacement programme. During the programme patients attended the bariatric clinic every two weeks for 24 weeks (14 visits in total), met the nurse, dietician and physician at each visit, had periodic blood tests performed and had weight, height and blood pressure measurements taken. All baseline and follow-up measures for the programme were conducted in the Bariatric Medicine Clinic at the Centre for Diabetes, Endocrinology and Metabolism in Galway University Hospitals (GUH).

2.2.2: Inclusion and Exclusion criteria:

Male and female patients aged 18 years or older, referred to the bariatric service for assessment of severe obesity were included in the study. Severe obesity was defined as a BMI ≥40 kg m^{-2} (or ≥35 kg m^{-2} with co-morbidities such as type 2 diabetes or obstructive sleep apnea syndrome). Patients must have been willing to attend all the 14 scheduled study visits. Females of childbearing potential who were pregnant, breast-feeding or planning to become pregnant or were not using effective contraceptive methods were not considered eligible for the programme. Patients with recent myocardial infarction (within six months), untreated arrhythmia, untreated left ventricular failure, recent cholelithiasis (within the past year), hepatic or renal dysfunction, type 1 diabetes, untreated major psychiatric disorders, eating disorders, cancer, previous bariatric surgery, a BMI <35 kg m^{-2} or those deemed unlikely to attend for the full programme (e.g. frequent clinic non-attendance) were not considered as participants to the programme.

2.2.3: Ethics approval:

The study was approved by the Galway University Hospitals' Central Research Ethics Committee in December 2017 (ref CA 1900). As the programme was part of standard clinical care for patients attending our service between 2013 and 2016 and was not a prospective research study, we did not prospectively obtain written informed consent from patients to use their data for research purposes. Considering recent changes in

European legislation regarding the use of personal data (the General Data Protection Regulation (GDPR)), we have only used data in this study from the subgroup of patients who agreed to this retrospectively and provided written informed consent.

2.2.4: Measurements:

Weight was measured on a Tanita® scale and height with a Seca® wall-mounted stadiometer, according to departmental standard operating procedures. Blood pressure was measured with an automated oscillometric device (Omron®) using a large cuff on the right arm, after participants had been seated quietly for five minutes. Three measures were recorded at one-minute intervals. A 12-lead electrocardiogram was performed to exclude occult ischemic heart disease or cardiac arrhythmia. Bloods were drawn after an overnight fast for glucose, renal and lipid profiles. All blood samples were processed locally in the Galway University Hospitals' Department of Clinical Biochemistry (certified to ISO 15189 2007 accreditation standard). HbA1c was measured with HPLC (Menarini® HA8160 auto-analyzer). Total cholesterol was measured using the CHOP-PAP method. HDL-cholesterol and triglycerides were measured using the enzymatic and the GPO-PAP methods, respectively (COBAS® 8000 modular analyzer). Low-density lipoprotein (LDL)-Cholesterol was derived with the Friedewald equation. Information relating to antihypertensive, lipid lowering, and antidiabetic medication use at baseline and at the end of 24 weeks was extracted from the medical records of each participant.

2.2.5: Intervention:

The milk-based LELD consisted of three continuous eight-week phases (Figure 2.2.5.1), each with fortnightly visits to the bariatric medicine clinic. During the first (weight loss) phase from weeks one to eight inclusive, an exclusively milk-based liquid diet was prescribed, consisting of approximately 2.5 liters per day of semi skimmed milk divided in seven portions throughout the day in equal doses, with additional sodium replacement, vitamin, mineral and fiber supplementation, equating to approximately 1200 kcal/day. The precise caloric content and volume of milk was determined by baseline body weight, according to a departmental standard operating procedure. Throughout this phase, renal and liver profiles were assessed every two weeks and the patient was seen by the consultant endocrinologist, bariatric nurse and dietitian at each visit. During the second phase (weight stabilization) from weeks nine to sixteen inclusive, there was a gradual re-introduction of low-calorie meals from a set menu over eight

weeks, according to protocol under the supervision of the bariatric dietitian with fortnightly visits continuing. During the third phase (weight maintenance) from weeks 17 to 24 inclusive, the liquid component of the diet was stopped completely and a fully solid isocaloric diet was restarted, based on individualized meal plans, under the supervision of the bariatric dietitian.

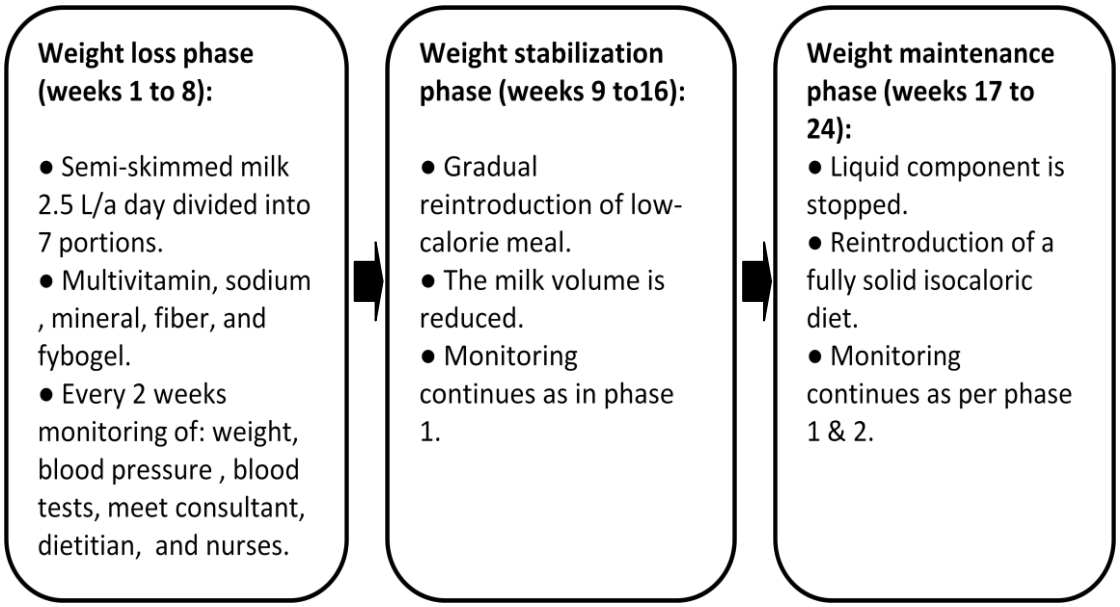

Figure 2.2.5.1: Overview of 3 phases of Milk-Based Meal Replacement Programme.

2.2.6: Outcome Measures:

The primary outcome measure was body weight. Within the cohort, there were distinct subgroups of patients for whom specific outcomes were more relevant, such as those with type 2 diabetes. There were several secondary outcome variables, including BMI, percentage excess body weight, blood pressure, HbA1c, and lipid profiles. We set the threshold for an elevated HbA1c ("high HbA1c") as "yes" if HbA1c ≥ 48 mmol/mol. We derived an *a priori* categorical variable for prevalent "dyslipidaemia" as "yes" if LDL-Cholesterol was ≥1.8 mmol/l in patients with diabetes, or ≥3.0 mmol/l in patients without diabetes at baseline, based on European Society of Cardiology guidelines. [125] Then we defined the presence of poor blood pressure control ("hypertensive") as "yes" if the systolic blood pressure (SBP) was ≥150 mmHg (in patients ≥60 years) or ≥140 mmHg (in patients <60 years) or if the diastolic blood pressure (DBP) was ≥ 90 mmHg (regardless of age). [126] Of note, we were unable to classify patients as having

hypertension or not as we did not prospectively record this in the medical notes in a consistent fashion, though we did record whether specific blood pressure medications were used at baseline and follow-up. We repeated the categorisation of all the above variables at each time point over 8, 16 and 24 weeks. Lastly, we derived a categorical variable "achieved 10% weight loss" as "yes" or "no" depending on whether the total percentage body weight loss was above this threshold at 8, 16 and 24 weeks.

2.2.7: Statistical methods:

Summary statistics (mean, standard deviation, range (or for categorical variables, the number, n and proportion, %)) for age, sex, height, diabetes status, weight, BMI, severe BMI status, % EBW, SBP, DBP, high HbA1c status, hypertensive status, dyslipidemia status, achieved 10% weight loss, total-, LDL-, HDL-cholesterol and triglyceride and HbA1c were obtained for times 0, 8, 16 and 24 weeks. We derived a surrogate measure of insulin resistance from the triglyceride: HDL-cholesterol ratio [127, 128]. Triglyceride: HDL-cholesterol ratio was shown to be the best predictor of insulin resistance and myocardial infarction in dyslipidemic patients who are at high risk of cardiovascular disease [127, 128]. In order to convert our mmol/l values to the equivalent United States values (mg/dl), we applied a conversion factor of 38.67 for HDL-cholesterol and 88.57 for triglycerides. Information on reasons for withdrawal from the intervention was not routinely recorded.

Continuous explanatory variables were compared using the two-sample t-test or Mann Whitney Test as appropriate, while categorical explanatory variables were compared using the Person's Chi-Square test. For completers, repeated measures ANOVA was used to determine whether there were statistically significant changes over time in outcome measures. All analyses were performed using SPSS version 24.

2.3: Results:

Between January 2013 and Oct 2018, 260 patients were enrolled into the milk-based meal replacement programme at the Bariatric Medicine clinic in Galway University Hospitals. Of these, 139 (53.5%) completed all 24 weeks of the intervention, with 121 (46.5%) discontinuing the intervention. From 139 completers, 105 (75.5%) agreed to participate in this study and provided written informed consent. Given that 1867 new patients were seen in our bariatric service over the six-year study period, 13.9% of newly referred bariatric patients ultimately participated in our milk programme.

The baseline characteristics of the 105 patients who completed the intervention and consented to study participation are described in table 1. Of these, 56 (53.3%) were female and mean age was 51.1±11.2 (range 18-71.6) years. Obesity-related Comorbidities were prevalent, with 35.2% of patients diagnosed with diabetes, 61.9% treated for hypertension and 40.9% on lipid lowering therapy. Changes in anthropometric and metabolic characteristics in intervention completers at 8, 16 and 24 weeks are shown in table 2.3.1. There was a 22.9±9.5 kg reduction in weight as anticipated (Figure 2.3.1), with a reduction in BMI of 8.0±3.2 kg m^{-2} (*P*<0.001). The proportion of patients losing 10% or more of their body weight at weeks 8, 16 and 24 was 59 %, 87.6 % and 86.7%, respectively (*P*=0.002), the proportion loosing 15% or more was 11.4%, 43.8%, 48.6% respectively (P=0.002).

There were no statistically significant changes in the systolic or diastolic blood pressures over time but the number of completers taking antihypertensive therapy fell from 68 (64.7%) at baseline to 37 (35.2%) at 24 weeks , a reduction of 29.5% (*P*<0.001). Specifically, of 25 (28.5%) taking angiotensin receptor blockers at baseline, 15 (14.2%) remained on these at follow-up, a reduction of 40% (*P*<0.001).

Table2.3.1: Changes in anthropometric and metabolic variables over time in 105 patients completing the milk programme.

	Week 0	Week 8	Week 16	Week 24	P-value
Anthropometric characteristics					
Weight (Kg)	144 (27.6)	128.3 (25.2)	122.5 (24.4)	121.1 (25.0)	<0.001
BMI (kg/m^2)	50.6 (8.0)	45.1 (7.5)	43.1 (7.4)	42.6 (7.6)	<0.001
EBW (%)	102.5 (32.0)	80.4 (30.1)	72.4 (29.6)	70.4 (30.4)	<0.001
Severe Obesity: N (%)	102 (97.1)	90 (85.7)	75 (71.4)	68 (64.8)	0.017
Metabolic characteristics					
Systolic BP (mmhg)	127.5 (13.4)	123.3 (13.8)	124.2 (14.3)	122.9 (14.6)	0.073
Diastolic BP (mmhg)	70.0 (10.7)	69.4 (11.6)	68.9 (11.5)	70.7 (11.2)	0.348
Hba1c (mmol/mol)*	38.8 (3.7)	36.0 (3.9)	35.1 (3.2)	35.0 (3.4)	<0.001
Hba1c (mmol/mol)**	66.3 (13.0)	53.3 (14.0)	48.3 (13.7)	48.4 (13.5)	<0.001
ALT (i.u./L)	35.2 (25.4)	36.7 (20.7)	27.5 (12.1)	24.8 (13.2)	<0.001
Total Cholesterol (mmol/l)	4.6 (0.9)	3.9 (0.9)	4.2 (1.0)	4.4 (1.1)	<0.001
LDL-Cholesterol (mmol/l)	2.7 (0.8)	2.2 (0.8)	2.6 (0.9)	2.6 (0.9)	<0.001
High LDL-Cholesterol: N (%)	49 (46.7)	26 (24.8)	41 (39.0)	46 (43.8)	<0.001
HDL-Cholesterol (mmol/l)	1.2 (0.3)	1.1 (0.3)	1.1 (0.3)	1.2 (0.3)	<0.001
Triglycerides (mmol/l)	1.8 (0.7)	1.4 (0.5)	1.3 (0.5)	1.3 (0.5)	<0.001
Triglyceride:HDL ratio	3.7 (2.2)	3.0 (1.3)	2.8 (1.4)	2.6 (1.3)	<0.001

*patients with no history of type 2 diabetes **patients with history of type 2 diabetes.

Of 30 (26.4%) taking angiotensin converting enzyme inhibitors at baseline, 21 (20%) remained on these at follow-up, a reduction of 30.0% (*P*<0.001). Of 24 (22.9%) taking calcium channel blockers at baseline, 6 (5.7%) remained on these at follow-up, a reduction of 75% (*P*<0.001). Of 31 (29.5%) taking beta-blockers at baseline, 28 (26.7 %) remained on these at follow-up, a reduction of 9.7 % (*P*<0.001). Of 31 (29.5%) taking diuretics at baseline, 16 (15.2%) remained on these at follow-up, a reduction of 48.4% (*P*<0.001). Of 6 (5.7%) taking alpha-blockers at baseline, 2 (1.9%) remained on these at follow-up, a reduction of 66.7 % (*P*<0.001).

There were statistically and clinically significant improvements in all components of the lipid profile, though unexpectedly these were most pronounced at 8 weeks and were somewhat attenuated by 24 weeks as shown in table 1, despite the progressive weight loss observed during that time. Moreover, the reduction in the proportion of patients with elevated LDL cholesterol from 46.7 % to 43.8 % between baseline and follow up (*P*<0.001). We were careful to avoid either the introduction or cessation of any lipid lowering therapy during the duration of the intervention. Of 39 (37.1%) completers who were taking statin therapy at baseline, all but one continued this throughout the intervention, with four patients also taking ezetimibe at baseline and follow-up. The reduction in the triglyceride: HDL cholesterol ratio was consistent with an increase in insulin sensitivity with the intervention.

We noted a significant reduction in HbA1c over time in the 37 patients with diabetes, equivalent to a 16.3±13.6 mmol/mol reduction by 24 weeks, with an effective normalisation to the diagnostic threshold for diabetes of 48 mmol/mol (*P*<0.001). There was also a significant reduction in HbA1c in patients without prevalent diabetes. Of 10 patients requiring insulin at baseline, five (50%) had stopped it by 24 weeks (*P*<0.001). Of 15 patients taking sulphonylureas, 13 (86.7%) stopped these (*P*<0.001) while 14 (77.8%) of 18 patients taking GLP-1 receptor agonists had stopped these by 24 weeks (*P*<0.001). Similarly, seven of nine (77.8%) patients stopped dipeptidyl peptidase inhibitors (*P*<0.001), while one patient taking pioglitazone discontinued this at the start of the intervention. Three of six patients taking sodium glucose co-transporter 2 (SGLT2) inhibitor drugs remained on these throughout the intervention (*P*<0.001), while 35 of 38 patients remained on metformin throughout (*P*<0.001). The number of patients on two or more antidiabetic medications came down from 32 to 9, a reduction of 71.9 %

(*P*<0.001). In the five patients who remained on insulin, their dose came down from 123.1±21.6 to 28.7±13.4 units per day (*P*<0.001), a reduction of 76.7%.

In patients with diabetes who completed the programme were slightly older and with a lesser degree of obesity than completers without diabetes. We observed a worse lipid profile in patients without diabetes, which we think may be due to a higher prevalence of statin use in patients with diabetes versus those patients without diabetes (64.9 vs 22.1 %, P<0.001). Notwithstanding the differences in adiposity at baseline, there was no difference in the anthropometric response to the intervention in completers with versus those without diabetes, as shown in figures 2.3.1-2.3.4.

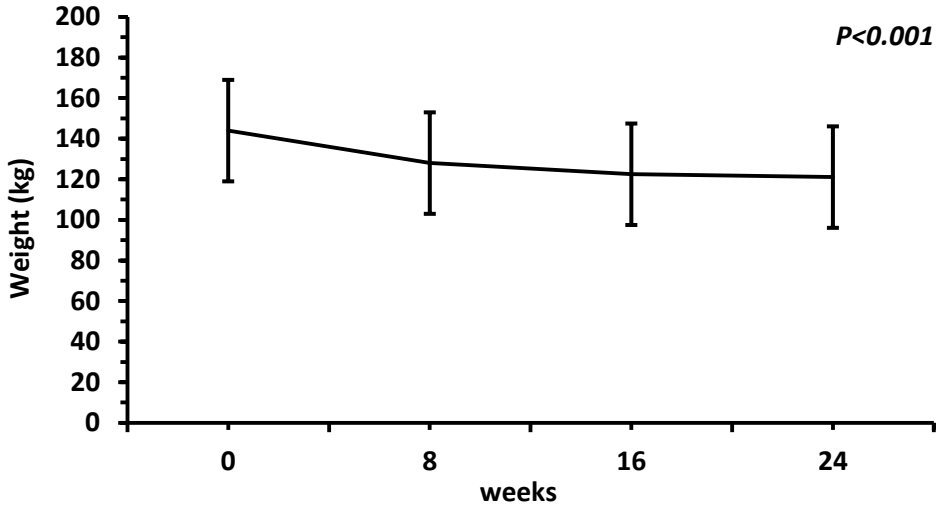

Figure 2.3.1a: Changes in weight over 24 weeks. *P is significant for the trend.*

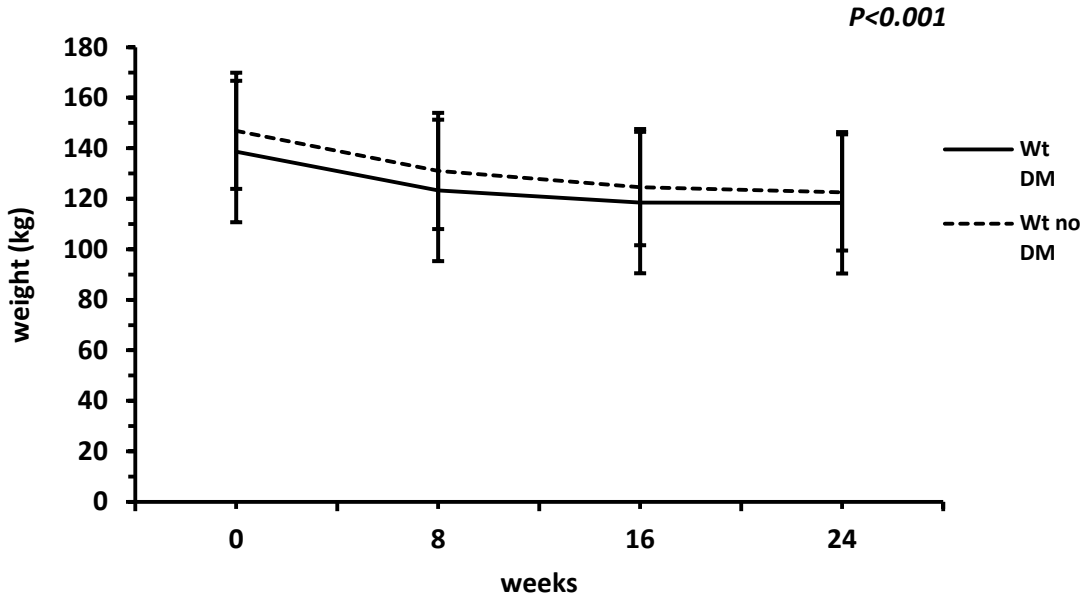

Figure 2.3.1b: Changes in weight by DM history. *P is significant for the trend.*

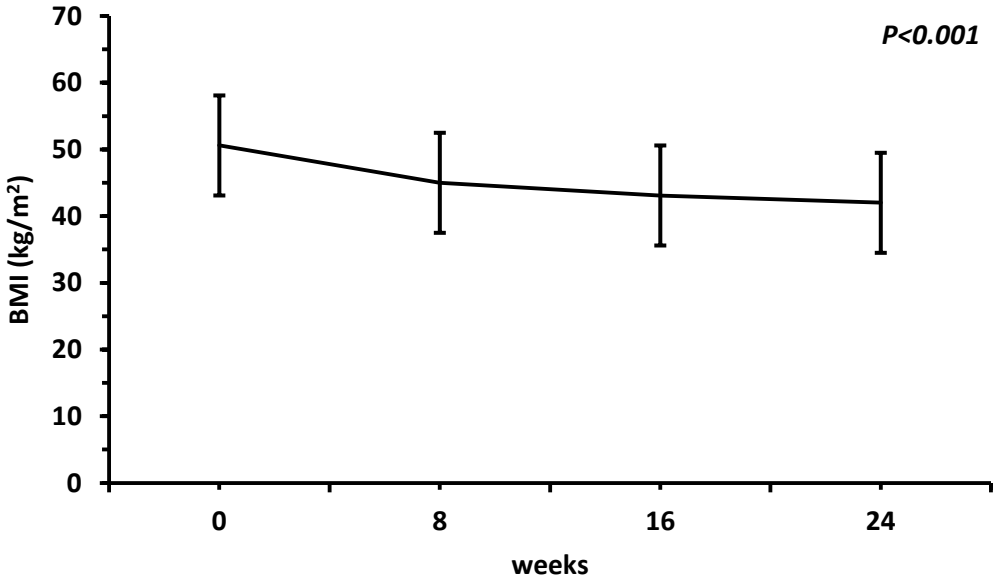

Figure 2.3.2a: Changes in BMI over 24 weeks. *P is significant for the trend.*

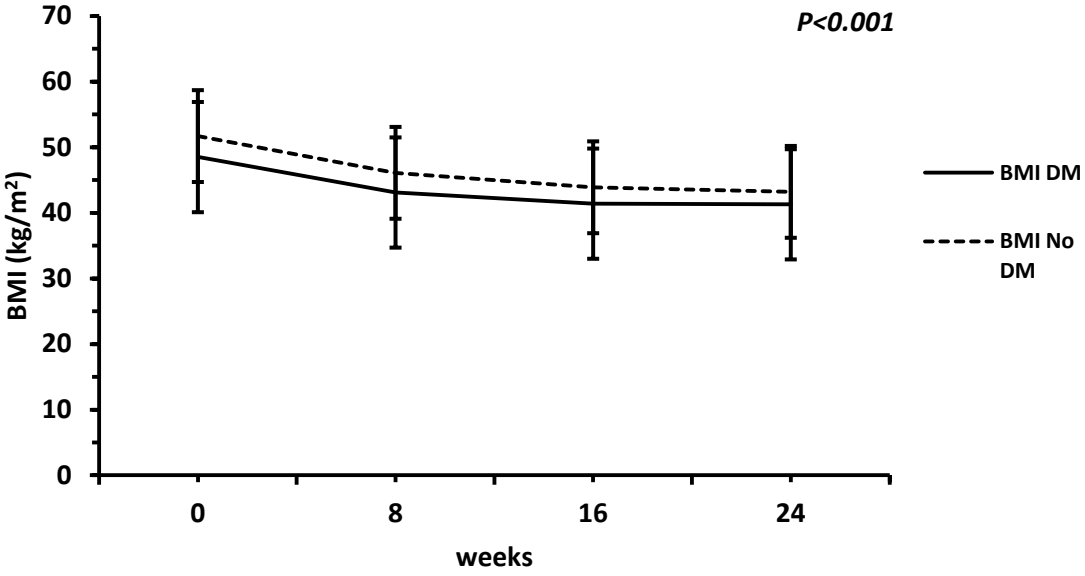

Figure 2.3.2b: Changes in BMI by DM history. *P is significant for the trend.*

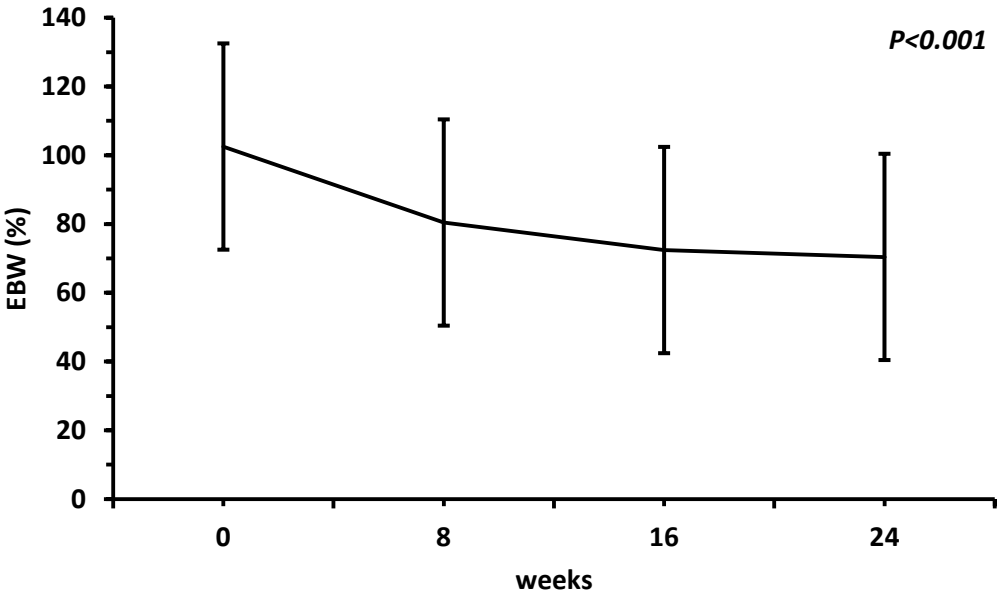

Figure 2.3.3a: Changes in EBW% over 24 weeks. *P is significant for the trend.*

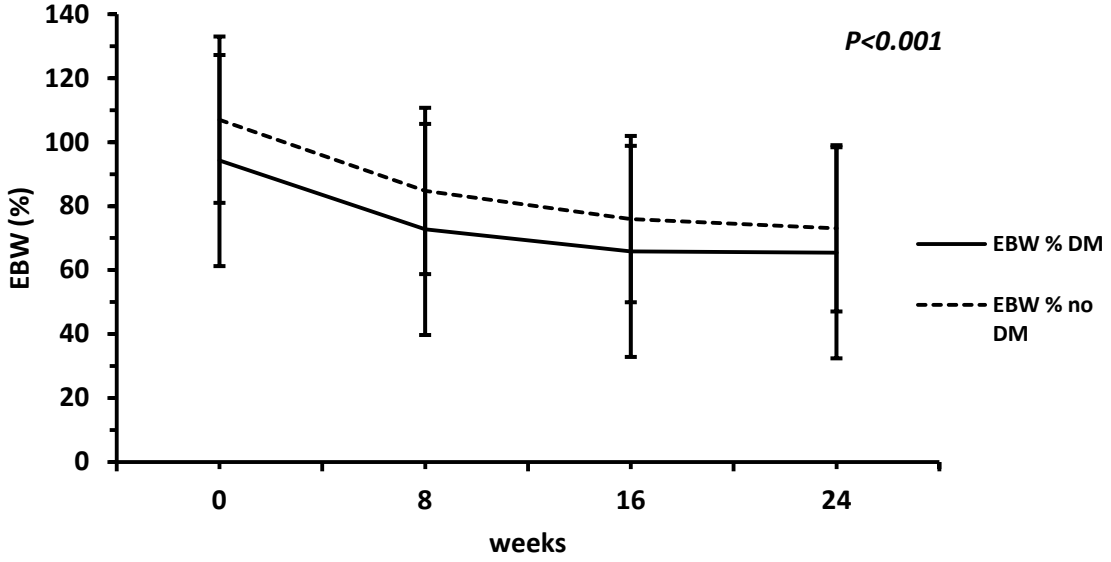

Figure 2.3.3b: Changes in EBW% by DM history. *P is significant for the trend.*

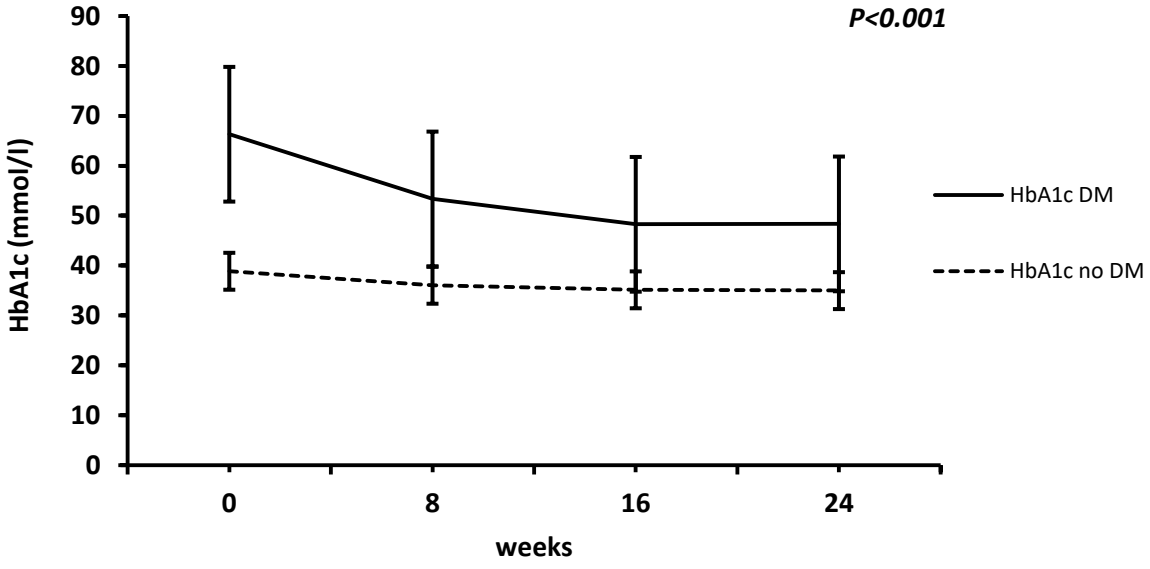

Figure 2.3.4: Changes in Hba1c over 24 weeks. *P is significant for the trend.*

2.4: Discussion:

This study showed significant improvements in excess weight and associated anthro-metabolic characteristics. These changes were significantly higher than those observed with a more conventional lifestyle modification programme which is utilized in a similar patient population, where mean weight loss in completers of a ten-week structured diet and physical activity programme was 2.7kg. [112]

Notwithstanding the relatively high drop-out rate, the fact that 54% of patients were able to tolerate the intensive schedule of clinic visits and the very significant curtailment of dietary intake, particularly in the first eight weeks, is surprising to many. The mechanisms underlying a likely reduction in appetite are unknown. Ketosis has been shown to attenuate the increases in ghrelin and appetite that occur with dietary restriction [129] and in recent years there has been a growing interest in the role of therapeutic ketosis in weight loss interventions. [130] Unfortunately, ketones are not routinely measured as part of the milk intervention, so their contribution to findings of this study is uncertain. These could and should be measured in future clinical trials of the intervention. It may be that the unique constituents of milk might account for some of the benefits seen in this intervention, though an aetiological trial with an equivalent non-milk-based meal replacement substrate would need to be done to clarify this issue.

I think that it is likely that we have underestimated the proportion of patients with dyslipidaemia in this cohort, because we have defined this only on the basis of the LDL-cholesterol level, using an arbitrarily defined threshold. We did not consider elevated total cholesterol or triglycerides or reduced HDL-cholesterol, but would normally do so in clinical practice in assessing a patient's lipid profile. However, I think that the large reductions in these individual components of the lipid profile that I have found are convincing and compelling, particularly given that statin therapy was neither initiated nor stopped during the intervention.

Arguably the most important limitation of this study is the lack of follow-up data for the patients who started the intervention but dropped out. However the focus of this study design was exclusively on patients who completed the intervention. Future trials will incorporate additional design elements to capture data on the duration of therapy intervention and dietary intervention on subjects that dropped out. It must be emphasised, therefore that this work is not a description of the effectiveness or efficacy

of the milk-programme but serves as a useful estimate of the effect size of the intervention as well as the attrition rate, to inform the development of future randomised controlled trials. It might be unlikely, but it is possible that some of those who dropped out may have had significant weight regain or serious adverse events that precluded continued participation, which were not pointed out, which is why there was no any attempt to compare data in completers and non-completers using last- or mean-observation carried forward analyses, for example. Nor was there evidence regarding the safety of the intervention, as this would require prospective collection of information as well as ongoing follow-up in patients who dropped out of the intervention. While participant retention in some studies is good[113, 131], attrition rates similar to ours here are not uncommon.[87, 116, 132] Thus, our per-protocol analyses of outcome changes in intervention completers is likely to have introduced some bias, residual confounding and possibly type 1 statistical error. However, even randomised controlled trials of meal replacement programmes have had methodological limitations. In one systematic review of 45 trials of non-surgical long-term weight loss maintenance interventions in adults with obesity, only 10 had robust allocation concealment, 17 described some form of blinding and 25 were deemed to handle incomplete data well[7]. Other reviews have confirmed that poor allocation concealment and blinding are particularly prevalent in trials of LELDs. [133] Hence, there is scope for enhanced rigour in similar future trials in order to reduce the potential for bias, residual confounding and type 1 errors. Clearly, it would be desirable to have information about safety outcomes and patients who drop out of the intervention in these trials, consistent with STROBE guidelines. [124]

2.5: Conclusions:

In spite of the limitations in this study, it is believed that the findings are novel and important particularly in the context of planning more robust prospective observational and randomised controlled trial assessment of the milk diet intervention. Such studies will need to extend the follow-up period well beyond the completion of the intervention, as weight regain is a well-established problem in the longer term. [110, 114] Ultimately this might help to broaden and refine the range of therapeutic options for adults affected by obesity.

Chapter three:

Changes in Alanine Aminotransferase in Adults with Severe and Complicated Obesity during a Milk-Based Meal Replacement Programme.

3.1: Background:

3.1.1: Alanine aminotransferase:

Alanine aminotransferase (ALT) is an enzyme found mainly in the cytosol of the liver and many other tissues, but ALT activities are much higher in liver than other tissues.[134-136] ALT is specific liver enzyme and elevated ALT activity in serum is rarely seen in conditions other than parenchymal liver pathology.[137] In the presence of cofactor pyridoxal phosphate, ALT catalyses reversible reaction of the transfer of the amino acid group of alanine to α-ketoglutarate, and finally resulted in generation of pyruvate which can be used for gluconeogensis or energy production via tricarboxylic acid cycle (TCA) , and glutamate which is responsible for production of nitrogen atoms for urea synthesis.[138] The plasma half-life of ALT is 18 hours.[136] The normal value of ALT ranges between 29 and 33 IU/L in men, and between 19 and 25 in women.[139, 140]

3.1.2: Effect of obesity and Non-alcoholic fatty liver disease:

Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are the most common cause of abnormally high ALT levels.[141-143] NAFLD is a medical term that describes a variety of histopathological changes occurring in the liver, including steatosis (fat accumulation within hepatocytes), and steatohepatitis (an inflammation of liver cells occurring as a consequence of abnormal hepatic fat accumulation).[144, 145] NAFLD and NASH are highly prevalent among bariatric patients. [146-149] In one study about half of recruited obese patients (32 out of 65) had elevated ALT and NAFLD.[150] In another study higher values of ALT was found to be an independent predictor for NAFLD, there was a significant association between higher ALT and NASH and fibrosis. [151] In the study aimed to determine the association between Intrahepatic fat (IHF) content, insulin resistance and abnormal energy homeostasis in 54 obese adolescents, about 30% of patients had NAFLD (IHF content > 5% wet weight),and this was associated with higher ALT (P= 0.006).[152] Another study showed that there was a significantly higher level of ALT at 30±4.41 (p<0.0001) among obese adolescents with reported high liver fat content.[153] In obese Korean children the prevalence of elevated ALT was 33.4% and 19.6% for boys and girls, respectively.ALT significantly correlated to BMI, waist circumference, lipid profiles, and blood pressure.[154]

3.1.3: Effect of bariatric surgeries:

Bariatric surgeries as shown in many studies in addition to improvements of comorbidities and successful long-term weight loss, they had also significant impact on fatty liver and ALT among obese patients.[155] In one study included males and diabetic patients with high ALT, Roux-en-Y Gartic Bypass (RYGB) and Laparoscopic Adjustable Gastric Banding (LAGB) both resulted in significant reduction in ALT three months postoperatively, and it remained at a low level for up to three years.[156] Another study comparing the effect of three types of bariatric surgeries [sleeve gastrectomy (SG), RYGB, and one-anastomosis gastric bypass (OAGB)] on inflammatory markers of 468 patients found that there was a significant reduction of ALT among three groups 6 months postoperatively.[157] NAFLD and NASH were both significantly improved two years after RYGB operation.[158] Another study was carried out in India to compare histological changes in the liver of severely obese participants before and after 6 months of bariatric surgery and showed that there was a significant and rapid improvement in liver histology after 6 months postoperatively; there was also reduction in ALT levels.[159] A Similar result was shown in the study assessing the effects of bariatric surgery on liver function tests in patients with NAFLD. This study reported a large reduction in ALT levels especially in the first 6 months postoperatively.[160] A French study which sought to prospectively determine the biological and clinical effect of bariatric surgery in 109 severely obese patients with NASH found that there was a significant improvement in NASH in 85% of patients 1 year after surgery (95% confidence interval [CI]: 75.8%-92.2%), and also there was a significant improvement in BMI from 49.3±8.2 kg/m^{-2} before surgery to 37.4±6.9 kg/m^{-2} after surgery (*p<0.0001*). There was also a significant reduction in γ-glutamyletransferases from a median of 51 [34, 87] IU/L before bariatric surgery to a median of 23 [14, 33] IU/L, *p<0.01,* after bariatric surgery, and insulin resistance index levels were reduced from a mean of 3.6 ± 0.5 to a mean of 2.9 ± 0.5, *p<0.01.* Moreover, ALT levels significantly decreased from 52.1±25.7 iu/l to 25±20 iu/l (*p<0.0001*). In the histological samples analysed in this study, steatosis was shown in 60% (IQR, 40%-80%) of patients before surgery, but only 10% (IQR, 2.5%-21.3%) (*P<0.0001*) of patients post-surgically, and the mean NAFLD score decreased from 5 (IQR, 4-5) to 1 (IQR, 1-2) (*P<0.0001*) in these patients.[161] However, in some studies not all weight loss surgeries have positive impact on liver ALT enzyme. A comparison study between SG, RYGB, and omega-loop gastric bypass (OLGB) surgeries showed that although OLGB participants had the highest excess weight loss frequency

(84.5%±26.7%), compared to SG (78.5%± 26.0%), and RYGB patients (72.0%±26.5%) (p=<0.05), there was a significant increase in ALT levels (p= <0.001) at 1-year follow up from normal baseline value. This increase was in 10%, 5.2%, and 1.9% of OLGB, RYGB, and SG group, respectively.[162] Similarly, a study aimed to evaluate the impairment in the liver function after Roux-en-Y gastric bypass (RYGB) and One-anatomises gastric bypass (OAGB) in 10 obese patients with NAFLD showed that liver dysfunction was diagnosed 15 months after RYGB and OAGB surgeries. The impairment in the liver function developed due to either a significant weight loss or regain occurring after RYGB or OAGB. Steatosis and fibrosis increased from 50 % (5 out of 10 patients) before bariatric surgery to70 % (7 out of 10 patients) after bariatric surgery, and cirrhosis increased from 10% (1 out of 10 patients) before bariatric surgery to 30% (3 out of 10 patients) after bariatric surgery. Moreover, higher levels of transaminases occurred in 70% of patients, one patient required liver transplantation, and impairment of coagulation parameters, thrombocytopenia, and hypoalbuminemia occurred 80%, 70%, 100% of patients, respectively.[163]

3.1.4: Effect of weight loss medications:

Recently developed GLP-1 analogues medications such as liraglutide and semaglutide have been proven in some studies to be effective in decreasing ALT levels and improving hepatic steatosis.[164, 165] They are primarily utilized to mange type 2 diabetes [164] , however, they are effective in treating obesity and reducing weight.[166] Semaglutide decreased ALT in two clinical trials in obese patients with type 2 diabetes. Moreover, dose-dependent reduction in ALT levels occurred in the two trials, with a greater decrease at week 28, and remained stable after this period until termination of treatments at week 52 or week 104, despite the continuation of weight loss. The decrease in ALT was greater in the weight management trial.[166] A study aimed to determine the effectiveness of liraglutide compared to sitagliptin and pioglitazone in 82 NAFLD patients with type 2 diabetes found that there were improvements in ALT levels, fasting blood glucose, HbA1c, liver fibrosis, and liver inflammation among the three groups of patients.[167] Additionally, in patients with type2 DM liraglutide dose of 1.8 mg resulted in reduction of ALT to −8.20 as compared to −5.01 IU/L in placebo group; P = 0.003.[165] In one study canagliflozin at a dose of 100mg and 300mg was found to have a significant reduction in ALT levels (p < 0.001) compared to placebo or sitagliptin. Also, there was a significant association between the reduction in ALT levels

and weight loss and improvement in HbA1c levels.[168] Pioglitazone was also shown to improve hepatic injury and fibrosis.[169] A study showed significant improvements in ALT among liraglutide and orlistat groups.[170] In another study in intervention group orlistat has resulted in moderate weight loss and this was associated with improvement in ALT level.[171] Orlistat resulted in reversal of liver fat on ultrasound and improved ALT levels in NAFLD patients.[172] However, liver injury can rarely occur in patients using orlistat.[173] Other weight reducing medications such as phenteramine/topiramate, naltraxone/bupropion, and lorcaserin have been associated with improving ALT levels in obese patients.[174-176]

3.1.5: Effect of lifestyle and exercise:

Several studies have shown the benefit of lifestyle intervention and exercise on NAFLD and ALT. [177] A study was conducted to reassess metabolic outcomes in young obese patients who underwent a hospital-based lifestyle weight loss intervention, and showed a significant reduction in ALT among these participants.[178] Multidisciplinary lifestyle interventions resulted in a reduction in body mass index standard deviation score (BMI-SDS) and was concomitant with improvement in liver transaminase in obese children and adolescents.[178] A study of 22 obese individuals with NASH comparing lifestyle to meal replacement interventions on NASH showed a significant reduction in internal fat and hepatic lipid content in both intervention groups. There was a significant weight loss (meal replacement group: -6.4 (3.6) kg, $P < 0.01$; lifestyle change group-: -9.1 (10.4) kg, $P < 0.01$). The reduction in hepatic lipid was strongly correlated to decrease in ALT.[179] In a study, 30 patients with NAFLD were randomly assigned to intervention group (text massages and counseling on healthy life style) and the control group (only counseling on healthy diet and exercise). There was a significant decrease in ALT level (-12.5 IU/L, P= 0.035) in intervention group.[180] A study showed that energy restriction for 8 weeks in 11 patients with type 2 diabetes was associated with a significant reduction in ALT from 46 ± 7 U/L at baseline to 33 ± 3 U/ at week 8. [181] A Meta analysis of RCTs of 1075 NAFLD patients showed that exercise significantly improved ALT and AST (P=< 0.05).[182] Intrahepatic fat improved independent to weight change (standardized mean difference=-0.98, 95% CI: -1.30 to -0.66). Exercise with or without dietary intervention resulted in decrease serum liver enzymes and improved liver fat and histology. Interventions using exercise and diet together resulted in reduction in ALT levels (P<0.01) and improved NAFLD activity score (standardized mean difference=-0.61, 95%

CI: -1.09 to -0.13).[182] In another study, aerobic exercise was associated with significant reduction in intrahepatic lipid.[183] A meta-analysis of 19 RCTs aimed to evaluate the individual impact of exercise and/or dietary interventions on ALT and other parameters in patients with NAFLD, Found that exercise and/or dietary interventions resulted in significant improvements in ALT levels as compared to control group.[184] Similarly, a study of 100 obese patients with NASH evaluating the effect of aerobic exercise combined with diet on biochemical parameters showed a significant improvement in ALT.[185] However, a recent meta-analysis of 28 trials found that physical activity independent to change in diet was significantly associated with intrahepatic fat content reduction (standardized mean difference, -0.69; 95% CI, -0.90 to -0.48), and reduction in ALT levels (mean difference, -3.30 IU/L; 95% CI, 5.57 to -1.04).[177]

3.1.6: Effect of dietary interventions:

Dietary fibre, hypocaloric, low fat and low carbohydrate diets:

Several dietary interventions have been shown to be beneficial in improving liver enzymes and intrahepatic fat**.**[186, 187] A study showed that increase intake of dietary fiber is associated with reduction in liver enzymes and improvement of steatosis.[188] Another study showed that weight loss induced by hypocaloric diet was associated with a significant reduction in serum transaminase levels and insulin resistance in obese patients with NAFLD. [189] A study examining two types of hypocaloric diets on patients with and without NAFLD showed that after patients underwent these diets there was a significant reduction in weight, liver transaminase in NAFLD group, HOMA and insulin level decreased, total cholesterol, LDL, and SBP. Weight loss secondary to low fat and low carbohydrate diets was associated with reduction of both liver transaminases, and insulin resistance in patients with NAFLD.[190] A study examining the effect of two types of hypocaloric diets on two groups of obese patients with (those with elevated baseline ALT levels) or without NAFLD (those with normal baseline ALT) showed that both diets resulted in improvements in all parameters, and liver enzymes including ALT in NAFLD group. Weight loss induced by two diets was associated with improvements in insulin resistance and elevated liver transaminases in patients diagnosed with NAFLD. [191] The reason for elevated baseline ALT was not clear in this study. [191]

Fructose restriction and dietary approaches to stop diabetes (DASH):

A study of obese children conducted to examine the effect of fructose reduction on NAFLD, and other parameters showed that hepatic fat was significantly decreased after fructose restriction.[192] Similarly, another study found that restriction of fructose amounting to 4% of total energy intake for nine days in obese children, was significantly associated with a reduction of steatosis, visceral fat de novo lipogensis, and improved insulin sensitivity.[193] A study assessing diet induced weight loss in obese patients with and without NAFLD showed that although weight loss was similar in both groups, the patients with NAFLD had significantly improved steatosis, and ALT.[194] A RCT was carried out among overweight patients with NAFLD showed that adherence to dietary approaches to stop hypertension (DASH) diet for eight weeks in NAFLD patients has resulted in improvement in BMI, and ALT.[195]

Fast weight loss method, Mediterranean diet, and very low calorie diet (VLCD):

A Randomized Control Trial of 24 weeks duration showed that patients who received a fast weight loss method had significantly reduced weight for 8-10 weeks, and there was significant reduction in fat mass from baseline as compared to controls. There was also significant reduction in fibrosis and steatosis as shown on ultrasound and liver histological sample. [196] At 24 weeks, weight loss had resulted in improvement in ALT. [196] Another study showed that patients who underwent a Mediterranean diet and a Mediterranean lifestyle interventions had significant weight loss compared to controls. The Mediterranean lifestyle group had significant reduction in ALT levels and liver stiffness compared to controls. [197] A systematic review showed that omega 3 reduced intrahepatic fat contents, and probiotics had mixed results. Low glycemic diet and diet low in fat had the same effect on reducing fatty liver and liver transamenase enzymes. [198] However, in one study patients who received VLCD (Very Low Calorie Diet) before bariatric surgery developed significantly higher levels of ALT.[199] In obese and overweight patients, drinking sugar-sweetened beverage was found to be associated with greater risk of fatty liver disease.[200]

Taken together, the studies performed on ALT and weight loss interventions to date suggest a more complex relationship between ALT and deliberate weight loss than was previously reported. A significant limitation of many of the previous studies in this area, however, was the use of a single endpoint of ALT measurement at the conclusion of the

weight-loss intervention. In our own centre, we currently provide a 24-week milk-based dietary intervention programme to aid weight management. During this programme, patients are monitored intensively with clinical assessment and blood tests every 2 weeks. The primary goal of the present study, therefore, was to characterise the relationship between ALT and weight loss to a greater degree by examining sequential changes in levels of ALT at multiple timepoints throughout intensively monitored weight loss intervention in people with obesity.

3.2: Methods:

This was a retrospective cohort study examining changes in ALT and weight in bariatric patients (similar cohort as in chapter 2) during their completion of a 24 week milk-based meal replacement weight loss programme conducted within the Diabetes Day Centre of University Hospital Galway. ALT was quantitated using the Roche Cobas® 8000 enzymatic assay with spectrophotometric detection. The decrease in absorbance at 340nm is directly proportional to the concentration of ALT. Inter-assay precision at a mean ALT concentration of 28U/L, 121U/L and 210U/L was 5.4%, 1.6% and 2% respectively. All of the above measures were preformed fortnightly throughout the programme. Normal ALT ranges were taken from the ACG clinical guidelines for the evaluation of abnormal liver chemistries (29 to 33 iu/l in men, and 19 to 25 iu/l in women).[139]

SPSS version 25 was used for all statistical analyses. Summary data were presented as means and standard deviations for normally distributed data and medians and interquartile ranges for skewed data, while categorical variables were presented as numbers and percentages.

In addition to analyzing ALT levels across the entire cohort we also classified the patient population into two groups according to baseline ALT levels as 'normal' (patients with normal baseline ALT levels), and 'high' (patients with elevated baseline ALT levels). For baseline characteristics, the independent samples t-test, was used to compare normally distributed variables between groups, while non-normally distributed variables were compared using the Mann-Whitney U test. Pearson's chi square was used to compare proportions of categorical variables.

The Wilcoxon signed-rank test was used to compare changes in ALT levels from baseline to week 2, and from baseline to week 24, respectively. Changes in ALT overtime in weeks 0, 2, 4, 6, 8, 16, and 24 were analyzed using the Friedman test. Changes over time in weight, BMI, excess body weight percentage (EBW%), HbA1c, total, LDL and HDL cholesterol and triglycerides were analysed using repeated measures ANOVA.

Correlation between Δ ALT concentration and Δ weight loss:

Δ weight from week 24 to week 0 was calculated as follow: (Δ weight 24-0 weeks= weight at 24 week - weight at 0 week). Δ weight from week 8 to week 0 was calculated as follow: (Δ weight 8-0 weeks= weight at 8 week - weight at 0 week).

Δ ALT from week 24 to week 0 was calculated as follow: (Δ ALT 24-0 weeks= ALT at 24 week – ALT at 0 week). Δ ALT from week 8 to week 0 was calculated as follow: (Δ ALT 8-0 weeks= ALT at 8 week – ALT at 0 week).

Because the data for Δ weight and Δ ALT was not normally distributed Spearman's correlation coefficient test was used for the analysis.

3.3: Results:

Baseline characteristics of study population:

Details of patients' characteristics are shown in Table 3.3.1. 105 patients with severe and complicated obesity were included in the study as before. The average age of the entire cohort was 51.2 (11.2) years. 56 (53.3%) were females. The median baseline ALT level was 28.0 [20.0, 40.5] iu/l. The cohort was also divided into two groups according to baseline ALT levels as normal or high. In the group of high ALT 23 out of 48 patients were females, while 33 out of 57 patients were females in normal ALT group, p= 0.307. The average age of participants with high baseline ALT was 49.1 (12.8) compared to 52.9 (9.4) for those with normal baseline ALT levels, p = 0.079. With the exception of ALT levels there were no statistically significant differences in other anthropometric and metabolic variables between the high and normal baseline ALT groups.

Changes in ALT, anthropometrics, and metabolic variables over 24 weeks:

Table 3.3.2 describes the changes in anthro-metabolic variables at weeks 2, 4, 6, 8, 16, and 24 of the programme. In general, all patients experienced a significant increase in ALT from baseline at median of 28.0 [20.0, 40.5] iu/l to 40.0 [26.0, 55.0] iu/l (p<0.0005), at week 2, then ALT seems to plateau at 40.0 [26.0, 60.0] iu/l in week 4, followed by a significant reduction to 35.0 [26.0,49.0] iu/l, 33.0 [23.0,42.0] iu/l, and 24.0 [19.0,35.0] iu/l (p <0.0005), in weeks 6, 8, and 16 ,respectively, reaching to 21.0 [17.0,28.3] iu/l (p<0.0005), by 24 weeks.(Figure 3.3.1a)

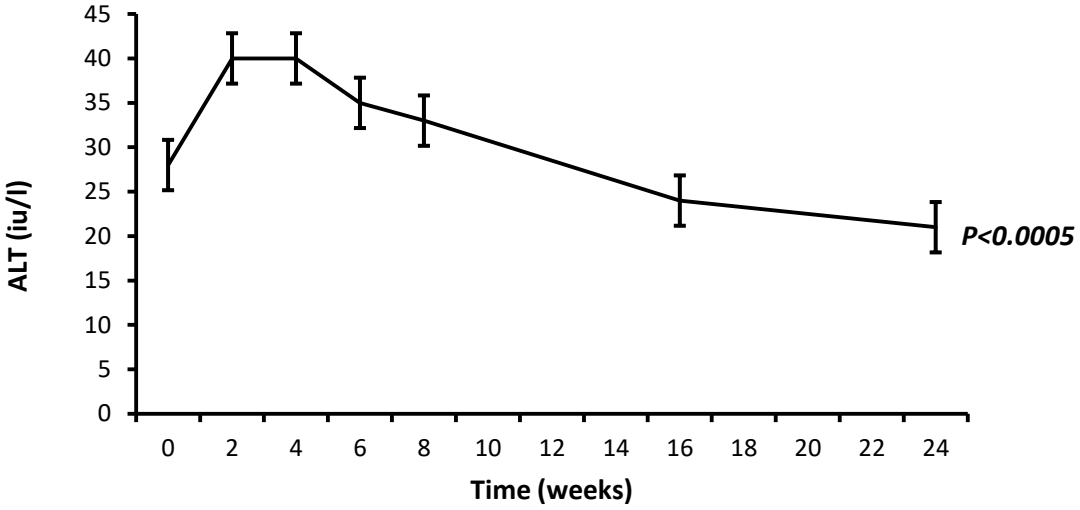

Figure 3.3.1a median ALT changes over 24 weeks in all patients.

When we further compared patients with normal ALT levels at baseline against those with elevated ALT levels at baseline we found that the rise in ALT levels across the first 8 weeks of the programme was greatest in those with elevated ALT at baseline ALT significantly increased in the high ALT group from 41.5 [34.3, 63.5] iu/l at baseline to 51.0 [41.0, 78.0] iu/l (p = 0.001), at week 2. It started to decrease at 46.0 [40.0, 66.0] iu/l in week 4 to 45.0 [34.5, 68.5] iu/l, 39.0 [32.5, 54.5] iu/l, and 35.0 [24.5, 42.5] iu/l (p <0.0005), in weeks 6, 8, and 16 respectively, reaching to 27.0 [21.0, 33.5] iu/l (p<0.0005), by 24 weeks. In contrast, the group with normal ALT levels at baseline only showed a non-significant increase in ALT from 22.0 [17.0, 24.5] iu/l at baseline to 28.0 [41.0, 21.0] iu/l, (p<0.0005), at week 2, decreasing to 28.5 [20.8, 43.3], 27.0 [21.8, 39.0], 26.0 [18.8, 36.0], and 20.0 [17.0, 25.0] iu/l, (p<0.0005), at weeks 4, 6, 8, 16, respectively, reaching to 19.0 [16.0, 22.0], (p= 0.008), by 24 weeks. (Figure 3.3.1b)

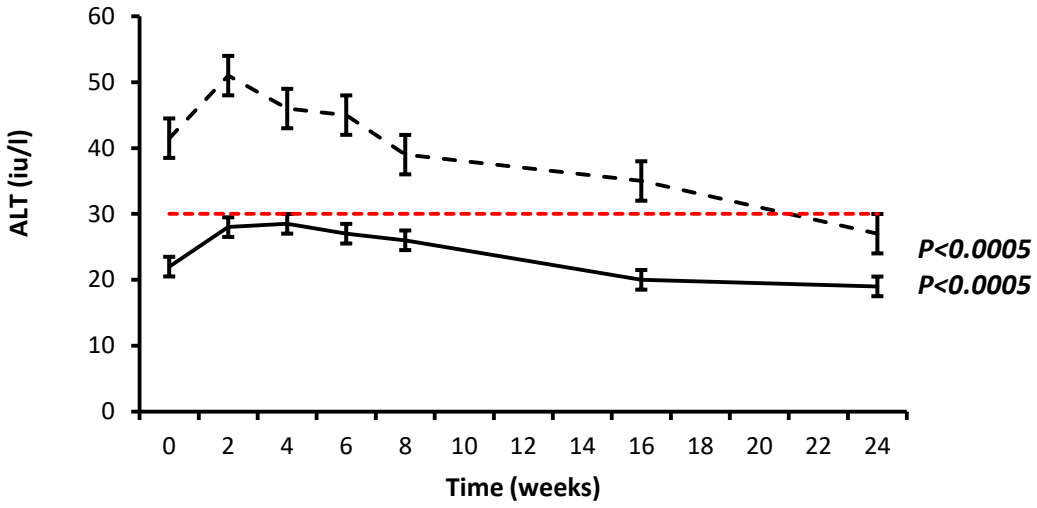

Figure 3.3.1b median ALT changes over 24 weeks in subgroups (dashed line for high ALT group. Continuous line for normal ALT group).

With regards to weight, the entire cohort showed significant reduction in weight from 144.2 (28.0) kg at baseline to 121.6 (25.4) kg, (p<0.0005), at 24 weeks. (Table 3.3.2)

Correlation between Δ ALT concentration and Δ weight :

From table 3.3.3, figure 3.3.2 and figure 3.3.3, it can be clearly seen that there is an inverse correlation between the magnitude of weight loss and the magnitude of ALT from week 8 to week 0 in all patients and more specifically in those with high baseline ALT (r_s = -0.307, p = 0.001 for all patients, and r_s = -0.423 ,p = 0.003 for high ALT group). The more weight loss, the higher ALT concentration.

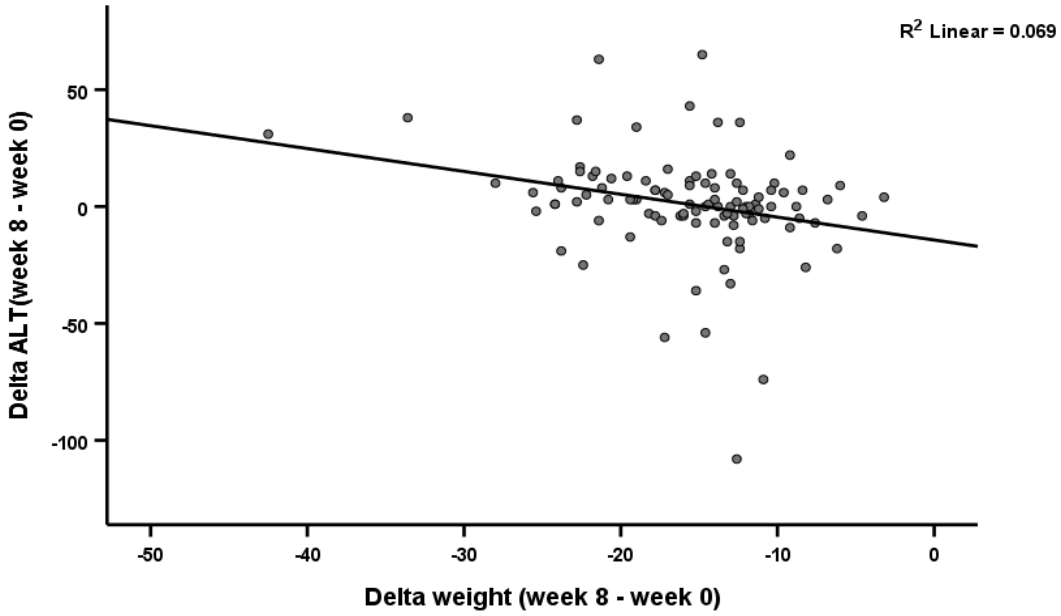

Figure 3.3.2 Correlation between delta ALT and delta weight from week 8 to week 0 in all patients (N = 105). *P = 0.001.*

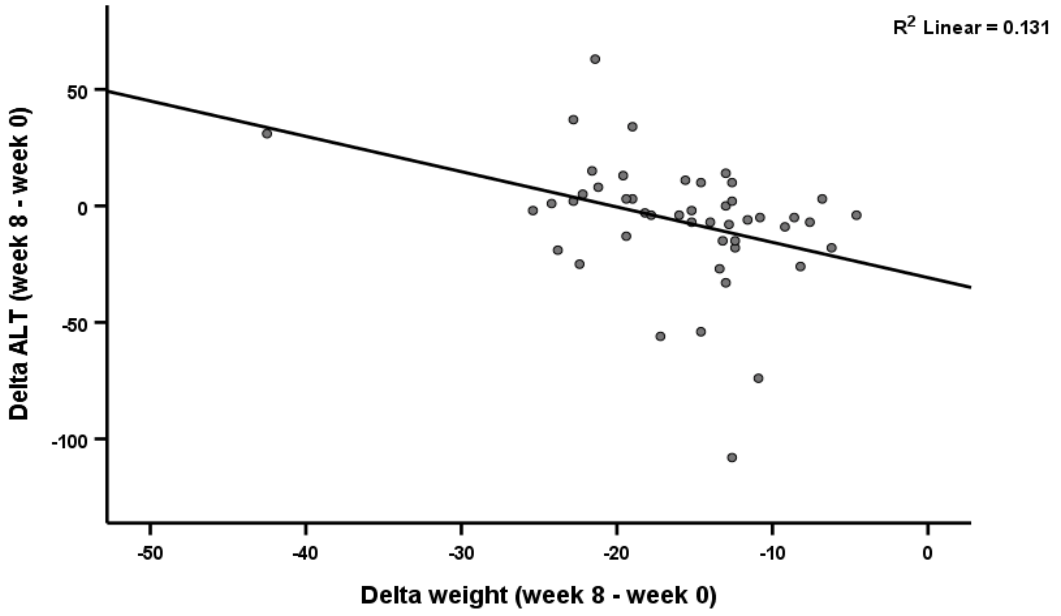

Figure 3.3.3 Correlation between delta ALT and delta weight from week 8 to week 0 in high ALT group (N = 48). *P = 0.003.*

Table 3.3.1 Baseline characteristics of patients completing the milk programme.

Parameters	All patients	Normal ALT	High ALT	*P*
N (%)	105 (100%)	57 (54.3%)	48 (45.7%)	
Female	56 (53.3 %)	33 (57.9 %)	23 (47.9 %)	0.307
Age (years)	51.1±11.2	52.9±9.4	49.1±12.8	0.079
Height (m)	1.7±0.1	1.7±0.1	1.7±0.1	0.502
Weight (kg)	144.0±27.6	145.2±27.3	142.6±28.2	0.627
BMI (kg m^2)	50.6±8.0	51.5±8.2	49.7±8.0	0.254
EBW%	102.5±32.0	106.0±33.0	98.6±31.2	0.254
ALT* (iu/l)	28.0 [20.0,40.5]	22.0 [17.0,24.5]	41.5 [34.3,63.5]	<0.0005
Total cholesterol (mmol/l)	4.6±1.0	4.6±1.0	4.6±1.0	0.786
LDL (mmol/l)	2.5±0.9	2.5±0.9	2.6±0.8	0.442
HDL (mmol/l)	1.2±0.4	1.3±0.4	1.2±0.4	0.131
Triglyceride (mmol/l)	1.8±0.8	1.7±0.6	1.9±1.0	0.151
Diabetes	37 (35.2 %)	19 (33.3 %)	18 (37.5 %)	0.656
HbA1c (mmol/mol)	48.2±15.6	48.1±16.3	48.4±15.0	0.943
Medications				
Metformin	38 (36.2 %)	22 (38.6 %)	16 (33.3 %)	0.576
Insulin	10 (9.5 %)	7 (12.3 %)	3 (6.3 %)	0.294
SU	15 (14.3 %)	10 (17.5 %)	5 (10.4 %)	0.298
DPP4	9 (8.6 %)	6 (10.5 %)	3 (6.3 %)	0.436
SGLT2	6 (5.7 %)	3 (5.3 %)	3 (6.3 %)	0.828
GLP1	18 (17.1 %)	9 (15.8 %)	9 (18.8 %)	0.688
PPARG	1 (1 %)	1 (1.8 %)	-	0.356
ARBs	25 (23.8 %)	12 (21.1 %)	13 (27.1 %)	0.470
ACEIs	30 (28.6 %)	19 (33.3 %)	11 (22.9 %)	0.239
Beta Blockers	31 (29.5 %)	17 (29.8 %)	14(29.2 %)	0.914
Alpha blockers	6 (5.7 %)	3 (5.3 %)	3 (6.3 %)	0.828
Diuretics	31 (29.5 %)	15 (26.3 %)	16 (33.3 %)	0.432
Ezetemibe	4 (3.8 %)	2 (3.4 %)	2 (4.2 %)	0.861

Values are presented as Mean ± SD.

**Denotes variables that are not normally distributed presented as median [IQR].*

Comparisons between groups were made using Independent samples t-test for normally distributed data, and Mann-Whitney U test for non-normally distributed data.

Proportions with categorical variables were compared using Pearson's Chi-square test.

BMI= Body Mass Index, EBW%= Excess Body Weight Percentage, ALT= Alanine Aminotransferase, LDL= Low Density Lipoprotein, HDL= High Density Lipoprotein, HbA1c= Glycated haemoglobin, SU= Sulfonylureas , DDP4= Dipeptidyl peptidase-4 inhibitors, SGLT2= Sodium-glucose co-transporter 2 inhibitors, GLP1= Glucagon-like peptide 1, PPARG= Peroxisome proliferator-activated receptor gamma, ARBs= Angiotensin II receptor blockers and ACEIs= Angiotensin-converting enzyme inhibitors.

Table 3.3.2 Changes in anthropometric and metabolic variables over time in patients with normal and high baseline ALT completing the milk programme.

Parameters	Week 0	Week 2	Week 4	Week 6	Week 8	Week 16	Week 24	*p*
ALT (iu/l)*	25 [20.0,37.3]	38 [26.0,53.5]	39 [26.0,60.0]	35 [26.0,49.0]	32 [23.0,41.3]	24 [19.0,34.3]	21 [17.0,28.3]	<0.0005
ALT (iu/l)*$^{\Delta}$	41.0 [33.0,62.5]	50.0 [40.5,85.0]	50.5 [39.5,68.3]	41.0 [34.0,67.3]	39.0 [32.0,51.0]	34.5 [25.0,41.3]	26.5 [21.0,36.5]	<0.0005
ALT (iu/l)*$^{\#}$	20.5 [17.0,24.0]	29.5 [20.3,41.0]	28.5 [20.3,43.0]	27.0 [21.3,38.5]	26.0 [18.3,36.0]	20.0 [17.0,25.0]	19.0 [16.0,22.0]	<0.0005
Weight (kg)	144.2±28.0	137.6±27.4	134.1±27.0	131.0±26.2	128.3±25.6	123.0±25.0	121.6±25.4	<0.0005
BMI (kg m^2)	50.7±8.1	47.4±10.4	45.4±12.0	44.5±12.0	45.2±7.6	43.2±7.5	43.0±7.6	<0.0005
EBW (%)	103.1±32.4	89.6±42.0	81.6±48.2	78.3±47.4	81.0±30.6	73.0±30.0	71.0±31.0	<0.0005
HbA1c (mmol/mol)†	39.3±4.0	37.6±4.0	37.0±4.0	36.3±4.0	36.0±4.2	35.4±3.3	35.3±3.5	<0.0005
HbA1c (mmol/mol)‡	67.2±13.3	62.2±13.0	59.0±13.0	56.0±14.2	54.0±15.0	48.5±14.4	48.6±14.1	<0.0005
LDL (mmol/l)	2.6±1.0	2.2±1.0	2.1±0.7	2.2±1.0	2.2±1.0	2.5±1.0	2.6±1.0	<0.0005
HDL (mmol/l)	1.2±0.4	1.1±0.3	1.1±0.3	1.1±0.3	1.1±0.3	1.2±0.3	1.3±0.4	<0.0005
Triglyceride (mmol/l)	1.8±0.7	1.5±0.6	1.4± 0.5	1.4±0.5	1.3±0.5	1.3±0.5	1.3±0.5	<0.0005
Total cholesterol (mmol/l)	4.6±1.0	3.9±1.0	3.7±1.0	3.8±1.0	4.0±1.0	4.2±1.0	4.4±1.0	<0.0005

*Values are presented as Mean ± SD. *Denotes variables that are not normally distributed presented as median [IQR].*

$^{\Delta}$ *Denotes variables that represent values higher than normal at the start of the programme.*

$^{\#}$ *Denotes variables that represent values that were normal at the start of the programme.*

† *Denote patients without history of type 2 diabetes.* **‡***Denote patients with history of type 2 diabetes.*

Table 3.3.3 Correlation between Δ ALT concentration and Δ weight.

Variable	Delta ALT (week 8- week 0)		Delta ALT (week 24- week 0)	
	r_s	*P*	r_s	*P*
Delta weight (week 8 - week 0)*	-0.307	0.001	-	-
Delta weight (week 24 - week 0)*	-	-	-0.013	0.89
Delta weight (week 8 - week 0)**	-0.423	0.003	-	-
Delta weight (week 24 - week 0)**	-	-	-0.214	0.15
Delta weight (week 8 - week 0)***	-0.243	0.06	-	-
Delta weight (week 24 - week 0)***	-	-	0.075	0.58

**Denote delta weight in all cohort (N = 105).*

***Denote delta weight in high ALT group (N = 48).*

****Denote delta weight in normal ALT group (N = 57).*

r_s *= Spearman's Correlation Coefficient*

3.4: Discussion:

NAFLD is a common health problem in patients with obesity.[201] Early diagnosis and management is vital to prevent irreversible complications of this condition such as cirrhosis and fibrosis and their clinical consequences.[202] Weight loss and the maintenance of healthy lifestyle and diets are integral to the treatment and prevention of this condition. ALT is an important marker for liver damage that has been shown to be elevated in patients with obesity and in those with NAFLD and NASH in particular. [150] As noted previously, there are conflicting data on the response of ALT to varied weight-loss interventions. In this study we demonstrated a significant and rapid unexpected rise in ALT levels in the first 8 week of a milk-based meal replacement programme that was particularly emphasised in those with elevated ALT levels at baseline. To the best of our knowledge this biphasic ALT response has not previously been demonstrated following a weight loss intervention of this nature and via the analysis of multiple timepoints, and our findings suggest a potential for increased hepatic inflammation early in the course of a weight loss intervention followed by a long term reduction in same. However, it must be emphasised that this assertion is speculative, because ALT is regarded as a "modestly good at best" indicator of hepatocyte damage in patients with NAFLD.[150] Our findings also suggest that patients with elevated ALT (suggesting a degree of NAFLD) are at higher risk of additional hepatic inflammation, at least during the early stages of dietary restriction-based weight loss interventions.

With regards to similar studies to our own, we note that Schwenger et al. previously examined the effects of a VLCD and also reported increases in ALT levels in the immediate aftermath of this intervention. [199] Furthermore we note that registry-based analysis of ALT levels at one year after bariatric surgery also reported increases in ALT. [162] These studies differed somewhat from our own. The analysis of ALT levels post VLCD was based on a single post-intervention ALT measurement taken in the immediate aftermath of significant weight loss. The post-bariatric patient cohort was also based on analysis of a single post-intervention ALT measurement, and it is notable that weight loss was likely ongoing in this cohort at only 1 year post-op. In contrast to these approaches, our own study analyzed measurements of ALT at multiple timepoints during an acute weight loss phase and also during a subsequent period of weight stability. This approach allowed us to demonstrate both the rise in ALT during acute weight loss but also the transient nature of this rise and the subsequent reduction in ALT levels in the

aftermath of significant weight loss once weight trajectories had stabilised. Ultimately, our findings help to explain the conflicting data on ALT levels and weight loss interventions in the literature to date, suggesting that ALT rises during acute and significant weight loss but that once this phase is over and weight stabilises then ALT levels typically fall below baseline levels.

The exact mechanism by which ALT levels increase during the early weeks of a weight loss intervention are unclear and were unfortunately beyond the scope of the present study. It has previously been demonstrated that during fasting increased adipose-derived non-esterified fatty acid (NEFA) flux to the liver occurs, promoting intra-hepatic fat accumulation.[203-205] Increased intrahepatic fat, in turn, has been associated with increased generation of fatty acids within the liver via de novo lipogenesis. [206] In a similar manner, studies of male livers following 48 hours of fasting have reported evidence of significant triglyceride accumulation.[207] It may be hypothesized, therefore, that states of reduced caloric intake such as those achieved during the weight loss phase of our intervention are associated with an increase in intrahepatic fat concentrations, which in turn may contribute to increases in ALT levels and, potentially, NAFLD severity. Once caloric intake and weight has stabilised this effect may then decrease and intra-hepatic fat accumulation may reverse, leading to an ultimate decrease in ALT levels from baseline. Further studies incorporating hepatic imaging and/or biopsy are required to explore this hypothesis further.

Limitations of this study include its retrospective nature and our use of ALT levels as an indicator of NAFLD. The usage of liver ultrasound, Fibroscan and/or recognised scoring systems to identify or predict the presence of NAFLD with greater accuracy may have added to the strength of our research, but unfortunately this information was not gathered as part of routine clinical care in our milk-based programme and as such these data were not available for analysis. We do note, however, that a number of studies by various other groups have previously utilised ALT levels as a primary marker of the presence of NAFLD, and we also note that the inclusion and exclusion criteria for the milk-based meal replacement programme would have reduced the risk of confounding (for example from excess alcohol consumption), making it more likely that variations in ALT levels within our population reflected changes in hepatic fat accumulation and inflammation.[150, 154, 208] However, we were not able to control the other possible risk factors for elevated ALT such as medications, iron levels, hemochromatosis status, age,

menopausal status in females due to the retrospective nature of the study. These confounders can be eliminated in more robust prospective observational studies and randomized control trials. One other limitation is the duration of the study in that we only examined the change in ALT levels over a period of 6 months, and as such future studies could focus on longer-time points, in particular with regards to changes in ALT levels during and following weight regain.

3.5: Conclusion:

To conclude, in adults with severe and complicated obesity undergoing a milk-based meal replacement programme, we demonstrated a biphasic ALT response, with an initial significant rise in ALT levels over the 8 week weight loss period which was greatest in those with elevated ALT levels at baseline. This was followed by an overall reduction in ALT levels during the weight stabilisation and maintenance phases of our programme which concluded at 24 weeks. These results may be of particular relevance to patients with severe hepatic inflammation undergoing significant weight loss interventions, and we submit that the extent to which liver fat accumulation fluctuates with weight loss interventions warrants additional research in the future.

Chapter four:

Long-term Changes in Weight in Patients with Severe and Complicated Obesity after Completion of a Milk-Based Diet Programme.

4.1: Background:

Obesity is a rapidly growing and highly prevalent multisystem disease. [3] Obesity increases the risk of many co-morbid conditions [4, 5] that can decrease life expectancy [209], increase rates of disability [210], and impose significant economic burdens on patients [211-213]. Reducing weight via lifestyle, medical or surgical interventions can lead to significant improvements in various markers of health in patients with obesity [214], but weight regain over time remains a significant challenge.[7] Whether or not weight regain occurs following an intervention (and the degree to which it occurs) appears to vary depending on the specific intervention and the study population. For example, in the national weight control registry study (which focused on lifestyle intervention via diet and exercise) successful weight loss was achieved and maintained for over ten years. [215] On the other hand, a systematic review of 80 weight-loss-focused randomized clinical trials using 8 different types of non-surgical weight loss interventions (diet alone, diet and exercise, exercise alone, meal replacements, very-low-energy diets, weight-loss medications (orlistat and sibutramine), and advice alone) reported that although short-term (6 months) weight loss varying from 5% to 16% was observed in the majority of patients initially, after an additional 6 months significant weight regain occurred.[87] Furthermore, in the SCALE trial the use of liraglutide helped to maintain and even increase weight loss amongst those who had already achieved ≥ 5% weight reduction through a low calorie diet (LCD), but once liraglutide was stopped weight regain was seen to occur within 3 months of discontinuing the drug. [216] As might be expected, bariatric surgery is associated with less weight regain in comparison to many other interventions in the long-term [217], with the Swedish Obese Subjects (SOS) study reporting 18% weight loss achieved at 20 years after surgery compared to only 1% weight loss in the control group receiving usual care. [8] Ultimately, while the literature to date has identified weight regain as a significant problem the data are somewhat conflicting, with results appearing to depend on the precise intervention utilised in particular.

In our own bariatric centre in University Hospital Galway (UHG) we provide a 24-week milk-based low-energy liquid diet (LELD) programme for patients with severe and complicated obesity. During this outpatient programme, patients are monitored intensively with fortnightly clinical assessments and blood tests. As our group has

previously reported, during these 24 weeks patients typically demonstrate significant improvements in metabolic outcomes such as weight and glycaemia, with 86.7% and 48.6% of patients achieving ≥ 10% and ≥ 15% weight loss at 24 weeks, respectively.[218] These results compare favourably to other centres using similar protocols, such as was reported by the Cambridge milk-based intensive weight loss study in which 69% of participants achieved ≥ 10% weight loss over a similar time period.[219] What has yet to be investigated and reported, however, is to what degree weight regain may occur in the aftermath of a milk-based LELD despite the impressive short-term results of the programme. The primary goal of the present study, therefore, was to describe long-term changes in weight following the completion of a milk-based LELD in patients with severe and complicated obesity. The secondary goal of the study was to examine if any predictors of weight regain could be identified within our cohort.

4.2: Methods:

This was a single-centre long-term longitudinal retrospective cohort study of patients with obesity who completed 24 weeks of a milk-based LELD programme (similar cohort as in chapter 2). For the purposes of the present study, data were retrospectively gathered from those patients who completed the entire programme, had follow-up visits in our center and who provided consent for their data to be gathered and analyzed for this purpose. Data were acquired from both paper-based medical charts and hospital electronic databases. It should be noted that patients who completed the programme but who subsequently underwent bariatric surgery or started on medications for the treatment of obesity (e.g. glucagon-like peptide 1 therapies) were excluded from the study population to minimize confounding variables.

SPSS version 26 was used to carry out the analysis. Descriptive statistics were used to interpret demographic characteristics of patients. Categorical variables were presented as numbers and percentages, while continuous variables were presented as mean and standard deviation for normally distributed data, and median and interquartile range (IQR) for non-normally distributed data.

We divided patients into four groups according to how long (after completion of the milk-based programme) we had follow-up weight and clinical data available on them to enter in the analysis (These patients were followed up in one point in time after completion of the milk-based programme). Group I included those patients for whom follow-up weight data was available from between 6 - 18 months after completing the programme. Group II included those patients for whom follow-up data was available from between 18 - 30 months after completing the programme. Group III included patients with follow-up data from 30 - 42 months after the programme, and Group IV included those with data available after 42 months of follow up. To compare categorical variables between the four groups Pearson's chi-square test was used. Non-normally distributed variables were compared using nonparametric median test.

Paired sample t test was used to compare the mean differences in weight at baseline and 6 months, and from 6 months to the follow up period. We calculated the relative percentage of weight regain by using the following formula: % of relative weight regain = [(weight at follow up - weight at 6 months)/ (weight at 0 week - weight at 6

months)/]*100, and we reported median [IQR] using nonparametric median test. Linear regression was used to determine the predictors for weight regain.

4.3: Results:

Of the 105 patients who consented to data gathering and retrieval 78 were included in the final study population (we excluded from the analysis 17 patients who received GLP 1 therapy, 8 patients who received sleeve gastrectomy and 2 patients who received both). Of those 78 patients, 41 (52.6%), 17 (58.6%), 7 (46.7%), 11 (50.0%), 6 (50.0%) were female in the entire cohort, Group I, II, III, and IV, respectively.(Table4.3. 1) The mean age was 51.6 ± 12.0 years. At baseline the entire cohort had a mean weight of 144 ± 26 kg and a BMI of 50.5 ± 7.6 kg/m^{-2}.

The median follow up period were 23.2 [14.5, 37.5], 11.5 [6, 15], 22.4 [21, 25], 34 [32.2, 40.3], and 51 [47, 58] months for the entire cohort, Group I, II, III, and IV, respectively. As the duration of follow-up differed significantly within the study population the cohort was divided into one of four groups according to when the longest-term data was available on them subsequent to their completion of the milk-diet [Group I (6 - 18 months): n = 29 (37.2%), Group II (18 - 30 months): n = 15 (19.2%), Group III (30 - 42 months): n = 22 (28.2%), and Group IV (>42months): n = 12 (15.4%) patients] (Table4.3. 1).

The initial change in weight during the milk-based programme (from baseline to 24 weeks) was -22.5 [-20.4, -24.6; 95% CI] kg, $P<0.0001$. In the first and second years after the completion of the programme some weight regain was observed but this did not achieve statistical significance. The mean difference between weight at completion of the programme and at 1 year post-completion was 3.5 [-17.2, 10.1; 95% CI] kg, $p = 0.59$. Similarly the mean difference between weight at completion of the programme and weight at 2 years was 7.2 [-27.2, 12.7; 95% CI] kg, $p = 0.45$. However, weight regain in third, and fourth years proved to be statistically different. At 3 years participants had gained a mean weight of 15.9 [1.0, 30.8; 95% CI] kg, $p = 0.03$, while at 4 years they had gained a mean of 23.9 [5.2, 42.6; 95% CI] kg, $p = 0.01$, and were seen to return to their baseline weight (Tables 4.3.2, 4.3.3; figures 4.3.1, 4.3.2).

The median relative weight regain % at year 4 was 91.8% [58.8, 179.5], which was significantly different ($P = 0.017$) than the median weight regain in the 1st, 2nd and 3rd years of follow up which were 7.6% [-60.6, 86.7], 4.5% [-56.4, 132.3], 52.1% [-11.0, 164.0], respectively (Figure 4.3.3).

With regards to predictors of weight regain, we observed an inverse association between EBW% lost during the intervention and weight regain in the first and third years of follow up β=-2.6 [-4.8, -0.3] p=0.024, and β=-3.8 [-6.5, -1.0] p = 0.009, respectively (those who lost the most weight during the milk programme regained less weight during the first and third years of follow up). Age and gender were not associated with the risk of regain (Table 4.3.4).

4.4: Discussion:

As many patients who suffer from obesity will confirm, it is frequently not loss of weight that is the main challenge they face in managing their disease but instead it is maintaining that weight loss over the longer-term. The present study highlights that difficulty even in the aftermath of very significant weight loss achieved through a structured milk-based LELD programme with very frequent contacts between patients and the bariatric team. Over a follow-up period of 4 years, our data indicate that weight regain occurred amongst our participants (most notably in the 3rd and 4th years after the programme) such that, on average, all of the weight lost was regained at the 4 year mark. To the best of our knowledge the present study is one of the few to provide long-term data after an effective weight loss intervention in patients with severe and complicated obesity and it is the first to examine weight regain after a milk-based LELD programme.

As noted previously, our study is consistent with some if not all of the previous research into long-term weight regain, and highlights the observation that dietary interventions (even when intensive and highly effective from the initial weight loss perspective) do not match bariatric surgery results with regards to the maintenance of weight loss.[8, 87, 216] Even in the National Weight Control Registry (NWCR) were about 12% of participants remained obese after 5.2 years of follow up.[215] The reason for weight regain after successful weight loss is complex and likely multifactorial, with factors such as hypothalamic-mediated weight homeostasis, environmental and psychological variables, frequency of interactions with health care professionals, participation in exercise and underlying genetic pre-dispositions of the patients themselves to obesity all playing potential roles.[220-223] With regards to weight homeostasis, we note that studies of diet-induced obesity-defender rats have demonstrated that even after long-term calorie-restriction there is a potent neuro-hormonal response predisposing to weight regain to baseline once calorie restrictions are lifted.[224] In a similar fashion in humans it has been reported that weight loss achieved through very low calorie diet interventions leads to alterations in gut hormones that promote hunger and decrease satiety and that there is also a reduction in basal metabolic rate, changes that together promote weight regain until the person returns to their 'setpoint'.[28, 225] In terms of what can be done to counteract these physiological factors promoting weight regain, studies have suggested that the long-term adoption of behaviours that promote reducing energy intake and

increase energy expenditure (particularly self-monitoring of weight and eating, and cognitive/psychological behaviours such as self-efficacy for weight management and self-efficacy for exercise) may help to avoid regain. [226, 227] Long-term behaviour change, however, may be difficult to achieve, thus the problem of weight regain remains significant within the field of obesity.

With regards to predictors of weight regain within the present study, we report that patients who lost larger amounts of their EBW saw less regain in the 1st and 3rd years of follow-up. Whether this is a behavioural phenomenon (e.g. more observed weight loss motivated patients to a greater degree) or whether those patients were different at baseline in terms of their ability to lose weight and maintain said weight loss cannot be determined from the present study, but our results do suggest that greater initial weight loss may be associated with better weight loss maintenance. These findings are similar to observations from the National Weight Control Registry (NWCR) where those patients with greater initial reductions in body weight also saw better weight loss maintenance over the subsequent 10 years.[215] Thus our findings are consistent with those previously reported, albeit in the novel setting of the aftermath of a milk-based LELD programme, and suggest that greater initial weight loss during interventions may be a positive long-term indicator.

Our study has a number of important limitations including its retrospective nature and the wide range over which follow-up data were available. The use of groups based on the time period of their follow-up data provided a more accurate picture of weight regain over time when compared against a single average follow-up date for the entire cohort. However this also led to smaller sample sizes for analysis. Furthermore, we note that most of patients in this cohort also went through our Croi CLANN lifestyle modification programme either before or after their participation in the milk-programme [86], and it is possible that participation in this programme (which focuses on sustainable lifestyle change and modest weight loss) may have helped to decrease weight regain in the aftermath of the milk programme (the mean weight loss in Croi CLANN study was 2.7 Kg).[86] This speculation, however, does not detract from our primary observation that weight regain to baseline occurred by 4 years in our population. Finally, we note that we excluded patients who received GLP 1 agonist therapy bariatric surgery after completing the milk-based programme. While this

decreased the numbers of the entire cohort we felt that these were important potential confounding influences in terms of assessing long-term weight regain.

4.5: Conclusion:

To conclude, among patients with severe and complicated obesity who completed a milk-based LELD programme, significant initial weight loss was followed by substantial weight regain. Those that lost the most weight during the programme regained less weight during follow-up, but it is clear that weight regain remains a significant problem in the treatment of the chronic disease of obesity. These findings have important implications for our own (and other centres') clinical practice. We are in the process of translating these results into improved patient care. The changes that we are likely to make based on these findings include 1. Defining very strict entry criteria to include only those co-morbidities such as poorly controlled type 2 diabetes, chronic venous insufficiency which are likely to improve substantially with milk-based meal replacement and also introducing other weight loss strategies (such as drug therapy) earlier after completion of the programme, to augment weight loss maintenance.

Table 4.3.1 Characteristics of the follow-up groups.

Variable	Baseline values:	6-18 months	18-30 months	30-42months	>42 months	*P*
N (%)	78	29 (37.2%)	15 (19.2%)	22 (28.2%)	12 (15.4%)	
Sex (Female)	41 (52.6%)	17 (58.6 %)	7 (46.7 %)	11 (50.0 %)	6 (50.0 %)	0.867
Follow up period (month)*	23.2 [14.5, 37.5]	11.5 [6,15]	22.4 [21,25]	34 [32.2,40.3]	51 [47,58]	

**Denoted variables that are not normally distributed presented as median [IQR].*

Proportions with categorical variables were compared using Pearson's Chi-square test.

Comparisons between groups were made using nonparametric median test for non-normally distributed variables.

Table 4.3.2 Changes in weight from week 24 to follow up.

Follow up group	N (%)	Mean weight at 24 weeks	Mean weight at follow up	Mean[95%,CI] of weight regain	*P*
6-18 months	29 (37.2%)	118.7±23.6 kg	122.2±20.7 kg	3.5 [-17.2,10.1] kg	0.59
18-30 months	15 (19.2%)	117.6±19 kg	124.8±32.1 kg	7.2 [-27.2,12.7] kg	0.45
30-42 months	22 (28.2%)	121.4±23.6 kg	137.4±26.9 kg	15.9 [1.0, 30.8] kg	0.03
>42 months	12 (15.4%)	124.8±30.4 kg	148.7±44.9 kg	23.9 [5.2,42.6] kg	0.01

Paired sample t test was used for all analysis.

Table 4.3.3 Changes in weight from week 0 to follow up.

Follow up group	N (%)	Mean weight at week 0	Mean weight at follow up	Mean[95%,CI] of weight regain	*P*
6-18 months	29 (37.2%)	140.5.±24.4 kg	122.2±20.7 kg	-18.3 [-32,-4] kg	0.01
18-30 months	15 (19.2%)	143.0±25.0kg	124.8±32.1 kg	-18 [-40,4] kg	0.1
30-42 months	22 (28.2%)	143.0±23.0 kg	137.4±26.9 kg	-5 [-20.4, 9.2.] kg	0.44
>42 months	12 (15.4%)	147.4±33.0 kg	148.7±44.9 kg	1.2 [-14.5,17.2.] kg	0.86

Paired sample t test was used for all analysis.

Table 4.3.4 Analysis of possible predictors of weight regain.

Variable	β	R^2	p-value	95% CI
6-18 months:				
Sex (males and females)	-119.2	-0.33	0.135	[-279.1, 40.7]
Age (years)	0.97	0.06	0.771	[-5.9, 7.8]
EBW%	-2.6	0.24	0.024	[-4.8, -0.3]
TWL%	5.0	0.04	0.37	[-6.4, 16.4]
18-30 months:				
Sex (males and females)	-83.9	0.06	0.35	[-271,104]
Age (years)	2.8	0.03	0.48	[-5.6,11.3]
EBW%	-1.6	0.06	0.368	[-5.5,2.2]
TWL%	-3.6	0.01	0.69	[-23.2, 15.9]
30-42 months:				
Sex (males and females)	-80.9	0.03	0.39	[-273,111.2]
Age (years)	5.7	0.09	0.16	[-2.5, 14.0]
EBW%	-3.8	0.29	0.009	[-6.5,-1.0]
TWL%	6.0	0.03	0.43	[-9.7, 21.9]
42 months:				
Sex (males and females)	-49.5	0.03	0.60	[-256.2, 157.2]
Age (years)	-3.5	0.13	0.26	[-10.1,3.1]
EBW%	0.71	0.03	0.695	[-2.3, 3.8]
TWL%	7.7	0.09	0.35	[-10.1, 25.6]

EBW% = Percentage Excess Body Weight, TWL% = Percentage Total Weight Loss.

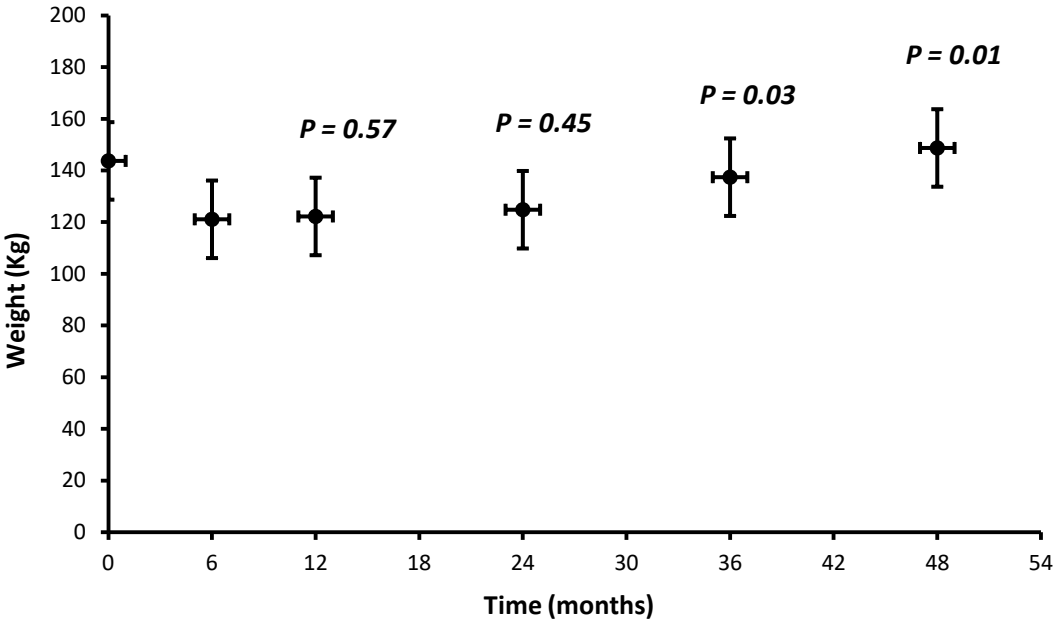

Figure 4.3.1: Long-term change in weight. *P values represent the changes in weight from the end of the milk diet (6 months) to the end of follow up period.*

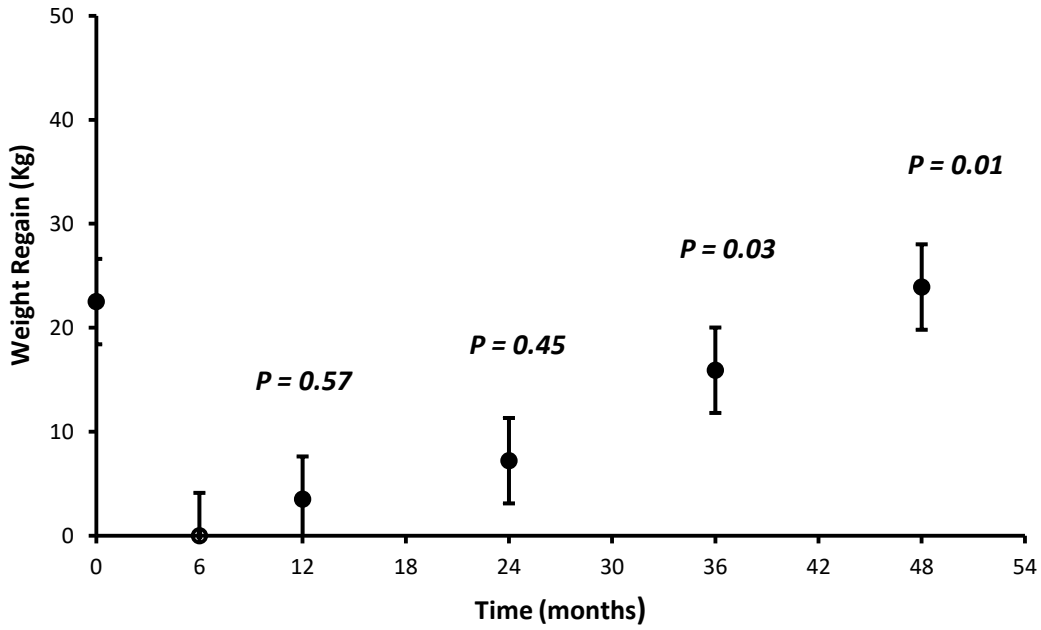

Figure 4.3.2: Mean differences of weight regain. *P values represent the difference in weight regain from the end of the milk diet (6 months) to the end of follow up period.*

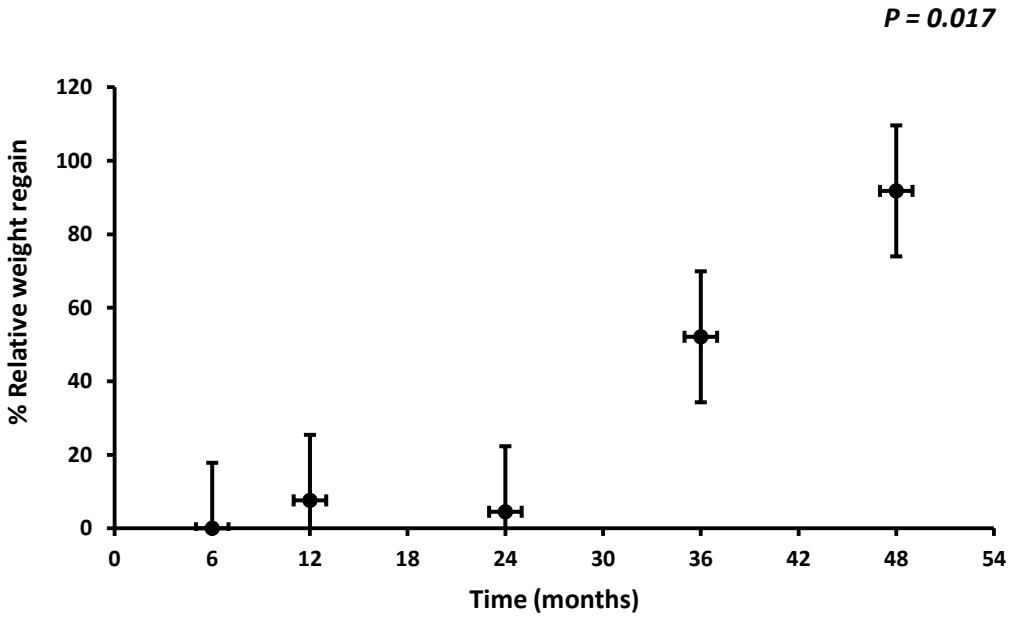

Figure 4.3.3: Relative percentage weight regain. *P value was reported from independent median test.*

Chapter Five:

Conclusions.

5.1: General Discussion and Conclusions:

In the bariatric clinic in Galway University Hospitals, the milk-based intensive weight management programme directed by multidisciplinary team including consultant endocrinologist, dietician, and nurses is prescribed as one of options to treat patients with severe obesity associated with acute severe complications such as poorly controlled type 2 diabetes, venous ulcers, degenerative disc disease or severe obstructive sleep apnoea. This thesis comprehensively examined the effect of a milk-based intensive weight management programme in several ways within these patients.

The benefit of a milk-based lifestyle programme in patients with severe and complicated obesity over 24 weeks was described in details in chapter two. According to the results of this study this programme has resulted in significant improvements in many outcomes such as excess body weight, HbA1c, and reduction in most of prescribed medications. Although, lipid profile notably improved in the first 8 weeks of the intervention, for unknown reason there was a dramatic increase in lipids after 24 weeks. Similarly, blood pressure did not change significantly after completion of this programme, but rather there was a significant reduction in percentages of patients who were taking antihypertensive medications by 29.5% *(P*<0.001). Based on our own early clinical experience in this intervention as well the experience of others we anticipated significant BP reduction in patients starting this intervention. Therefore we routinely titrated downwards blood pressure medication at early in the intervention and this often preceded reductions in BP.

The reason for doing so is because this is an outpatient intervention where patients were typically seen by a physician and a nurse every two weeks. In order to avoid a risk of significant hypotension which might have been debilitating or dangerous for patients we erred on the side of caution and chose to reduce their BP medications early. Thus we can surmise that in patients where BP readings remained the same but their requirement for antihyertension therapy diminished that the severity of their BP problem has been reduced.

86.7% of patients achieved ≥ 10% of weight loss, and 48.6% lost ≥ 15% after 24 weeks of the interventions, respectively. Similar to this programme is the Cambridge Intensive Weight Management Programme. In this programme, there were about 69% of patients achieved ≥ 10% body weight loss, but there was no statistically significant improvement in HbA1c, or blood pressure. [219]

NAFLD and NASH both are well known to be highly prevalent among patients with obesity. [146-149] In chapter three the effect of milk diet lifestyle intervention on ALT over 24 weeks among patients with severe and complicated obesity was assessed. In the first 8 weeks of milk diet there was an unexpected increase in ALT and was more pronounced in patients with a high baseline ALT but the reason for this increase was not determined. In the study examining the prevalence of simple steatosis and NASH in patients with obesity undergoing bariatric surgery post- very low calorie diet (VLCD), and assessing biochemical marker pre- and post VLCD found that although VLCD has resulted in significant improvements in BMI, fasting glucose and insulin, HbA1c, and lipid profiles, it was associated with a significant increase in liver transaminases. The mean difference in ALT before and after starting VLCD was 10.0(1.20, 18.80; 95% CI, *P*= 0.0276). [199] In another study, starting a low calorie diet (LCD) was immediately associated with significant increase in ALT in women, but not in men.[228] Also fasting may a play role in increasing ALT. During fasting increased adipose-derived NEFA flux to the liver can cause intrahepatic fat accumulation.[203-205] A recent study showed that patients with high liver fat had VLDL-FA generated from de novo lipogensis two times as high as those with low liver fat at 23.2%± 7.9% , and 10.1%± 6.7%, respectively; p<0.001. Moreover, patients with high liver fat had three times higher rates of increased de novo fatty acids synthesis than those with low liver fat (2.57 ± 1.53 μmol/min vs 0.78 ± 0.42 μmol/min; *P* = .001), and fasting lipogenesis was positively associated with interahepatic triglycerid levels in both patients groups (those with high liver fat and low liver fat). Elevated lipogenesis combined with excess adipose FA release, was felt to significantly correlate with risk of NAFLD.[206] Additionally, a study showed that there is an increase in NEFA (a marker of lipolysis) in adipose tissue after fasting in overweight and obese women.[229] Another study aimed to determine the changes in lean tissues triglyceride contents during fasting for 24 hours in healthy men and women, this study found that triglyceride accumulated in the livers of men, while there was a resistance of women to fasting-induced increases in hepatic triglyceride contents, suggesting that men are at higher risk to develop hepatic steatosis .[207] Fasting plasma concentration of NEFA was found to be strongly significantly correlated to the amount of visceral fat among individuals with normal insulin sensitivity (Pearson r =0.81, *p* = 0.03).[230]

In our study, although there was a significant association between weight loss and high ALT concentration during the first 8 weeks of the milk-based meal replacement diet, the significant reduction in ALT that was shown by the end of the programme was not

associated with overall weight loss (Table 3.3.3; figures 3.3.2; 3.3.3 in chapter three). A study examining the effect of two types of diets (low carbohydrate and low fat diets) on aminotransaminase showed that the reduction in aminotransferase was not associated with a type of diet, but rather was associated with weight loss.[231]

Long-term weight changes were determined in patients with severe and complicated obesity who completed 24 weeks of a Milk-based Intensive Weight Management Programme in chapter four. The results of this study showed that among patients with severe and complicated obesity who completed a milk-based meal replacement programme, after significant initial weight loss, weight regain was very substantial after two years of follow up. Moreover, those who lost the most weight during the milk programme regained less weight during 1st, and 3rd follow-up years. Yet, there is very significant lack of understanding of mechanistic basis behind longer-term weight regain after significant initial weight loss in most non-surgical weight loss interventions and the best example of this is the study of Franz et al. [87] From 2007 but it still as relevant now as it was published 13 years ago, and this study describes longer-term outcomes in patients who underwent intensive interventions at least 6 months with at least 2years outcomes data. This study showed that even the most intensive dietary interventions such as very low energy liquid diet or very low calorie diet which is typically be of order of 600 or 700 kcal/day, while the initial weight loss of about 18 kg can be achieved at 6 months, the challenge has been the relapse and weight regain as a role in these patients over a period of follow up after a year or two years and three years, and this probably is related to setpoint theory where patients cannot maintain lifestyle modification strategy for longer than a short period of time because the physiological changes occurred influenced their dietary pattern so they eat more again and they regain weight so this is a big challenge in the clinical practice and was something we were aware of.

The strength of this thesis is that through examining the effect of a milk-based meal replacement diet in a cohort of patients with severe and complicated obesity in short-term (6 months), I have shown that in chapter two of my thesis patients lost lots of weight, BMI, and EBW% and that their blood pressure medications reduced by 29.5%, and that HbA1c improved in those that had diabetes and that did not have diabetes at baseline. In those that had diabetes at baseline their HbA1c went from 67.2±13.3 to 48.6±14.1, $p<0.0005$. These significant results were incomparable to programme similar to ours which uses physical activity, medications and behavioural therapy in addition to

LELD as a treatment of obesity [219], the weight loss in this programme was lower than was achieved in our study, and there was no significant changes in HbA1c, blood pressure, and there was no available data for lipid profiles compared to our study. However, the limitations of chapter two of my thesis would be the retrospective nature of the study, and the second limitation would be the higher attrition rate where only 53.4% of patients who completed the study and the unavailability of the follow up data of those who dropped out. Therefore, future studies should include more robust and large prospective observational studies and randomized control trials to more understand and possibly tackle these limitations.

The strength of chapter three of my thesis is that I examined in short-term (duration of 6 months) the effects of a milk-based meal replacement diet in a similar cohort of patients on ALT in multiple timepoints, and I have shown that there was an unexpected and transient increase in the concentration of ALT in the 2nd, and 4th weeks of a milk diet, in both groups those they have high baseline ALT and those they have normal baseline ALT, and in those they have high baseline ALT the rise in ALT concentration in the 2nd and 4th weeks of the intervention was more pronounced, ultimately then they had a greater relative reduction in ALT in both groups by the end of 24 weeks. I have also shown that there was a moderate association between weight loss and the rise in ALT concentrations during the first 8 weeks of a milk diet, but there was no association between overall weight loss and the significant reduction in ALT concentration by the end of 24 weeks of the milk diet. I was not sure about the mechanistic basis of this observation, I have not confirmed that this was fatty liver, but I rather described the biochemistry changes, but this observation could be due to very rapid release of triglycerides from adipose tissues into circulation during a period of severe caloric restriction such we have with this programme, and these released triglycerides accumulate within the liver. There may be an element of liver injury associated with this intervention we do not know, but it may be that there is an inflammation in the liver as a result of this ingress of lots and lots of these fatty molecules. It may be that there are changes in ALT levels which again may be an indicator of fat accumulation within the liver that is so how related to adipocytes function not just release of the fuel and fat from adipocytes but also changes in leptin and adiponectin or other adipokines that are released from fat cells, and we know that fat cells integrate the link with inflammation in other tissues that is largely why there is a relationship between obesity and cardiovascular disease or obesity and diabetes.[232] So one of the things that we could do

to explore the mechanistic basis for this observation in the future studies would be better visualize the liver to confirm or refute the observation that fat accumulation within the liver changes during the early stage and late stage of the programme so, for example, we could use Fibroscan device for this or ultrasound device or magnetic resonance imaging (MRI) or we can even do a liver biopsy to best characterize the changes within the liver parenchyma overtime. The second limitation of chapter three of my thesis is that I examined these changes in short-term and the focus of the future studies should be exploring in more detail these changes in longer-term possibly by conducting a large observational study or even randomized control trial.

The strength of chapter four of my thesis is that I examined weight changes in long-term in the similar cohort of completers of the milk-based diet, and the strength of this study compared to the Cambridge study is that we have followed up patients over 4 years period, while in the Cambridge study they only had followed up patients for 3 months after completion of 6 months intervention and the result of this study was that after initial mean weight loss of 22.9±9.5 kg at 6 months of completion of the intervention, patients only lost about a mean of 1 kg at 3 months of follow up after completion of the programme. [219] In our study, however, although there was a minimal non-significant mean weight gain of 3.5 [-10.1, 17.2; 95% CI] kg, *P* = 0.59, at first year of follow up from initial weight loss, there still a significant weight loss of -18.3 [-32,-4; 95% CI] kg, *P* = 0.01, in the first year of follow up compared to baseline weight (Tables 4.3.2; 4.3.3 in chapter four). However, the limitations of the chapter four of my thesis could be the retrospective nature of this study, and the one point in time follow up resulted in the big gap in time between the completion of the programme and follow up. Secondly, I excluded from the study patients who had some definite interventions such as those who had sleeve gastrectomy or who were taking GLP 1 agonist or both of them; I excluded them from the study because I felt that they would have confounded the long-term follow up assessment. Moreover, the remaining number of included patients in the study was further divided into subgroups leaving each group with a smaller number of participants. Thirdly, most of the patients went through Croi ClANN programme. Though, I believe that this programme has minimal effect on these patients (they only lost a mean weight of 2.7 kg). [86] Finally, the significant weight regain that occurred in the 3rd and 4th years of follow up after initial weight loss. Though this regain was not significant when compared to baseline weight which means that patients went back to what they were previously. Whether this regain was associated with worse metabolic

profiles was not examined in this study. Therefore, the future studies should focus on evaluating weight regain including examining metabolic variables to see if significant improvements in these variables in short-term, as documented s chapter two of my thesis, remain within the normal limit or exaggerated to abnormal levels. This could be done in a large prospective randomized trial with regular follow up to understand both the weight regain and associated metabolic variables changes.

Overall, the results of this thesis will help to insure that appropriate statistical power calculations are included in future clinical trials study design using this type of intervention.

It is important to note that, the primary purpose of this programme is not only weight loss, but rather it aimed to be used as a treatment option for several serious medical conditions. For example, there have been significant improvements in metabolically sick patients when they underwent this programme. Also, there have been improvements in venous ulceration. Moreover, this programme is mandatory for those who are going to have bariatric surgeries such as laparoscopic sleeve gastrectomy in order to reduce the size of the liver before the operations. Also, it can be helpful to reduce weight for those who are going to have orthopaedic surgeries such as knee replacement or vertebral column operations. It can also be used to treat infertility in obese females diagnosed with polycystic ovary syndrome (PCOS); though, I have not examined this in this study. Therefore, the future studies would explore the effect of a milk-based meal replacement diet on infertility in obese females with PCOS and this possibly can be done in a prospective randomized control trial preferably in collaboration with gynaecologist specialists.

Appendices.

Protocol:

Intensive Weight Management Programme (IWMP)

Outline:

The Bariatric Medicine & Surgery (BMS) service in Galway University hospital provides a multidisciplinary team (MDT) approach for the management of severe obesity (BMI >40kg/m2). There are a variety of interventions that are open to patients attending the service including a diet & physical activity programme (CROI CLANN) as well as Bariatric surgery for suitable candidates; however these do not meet the needs of all patients. The aim of this intervention is to support patients in the improvement of medical risk factors through an intensive weight management programme over a period of 24 weeks.

This document outlines the standard operating procedures underlying the roles of the multidisciplinary team, which consists of consultant/registrar, obesity CNS & dietitian responsible for the management of the BMS service.

Definitions and abbreviations:

- Bariatric Medicine & Surgery (BMS)
- Galway University Hospital (GUH)
- Multidisciplinary Team (MDT)
- Body Mass Index (BMI)

General protocol overview:

Initial medical assessment

- Baseline bloods taken include, but are not restricted to: Urea & electrolytes, liver function tests (LFT), renal profile (RP), bone profile, fasting glucose, HbA1c, thyroid function tests (TFT), full blood count (FBC), fasting lipids, vitamin B_{12}, folate
- Patient is commenced on other medication as appropriate (e.g. anti-hypertensives, nicotine patches).
- The need for oral hypoglycaemic agents or insulin in patients with Type 2 diabetes is monitored. As a general guide insulin sensitisers (e.g. metformin) may be continued. However if the patient is currently taking sulphonylureas (e.g. diamicron MR), insulin secretagogues (e.g. replagnanide) or insulin, the dose should be reduced by 50% as soon as the patient commences the liquid low calorie diet. Blood glucose should then be monitored pre-meal and at bedtime (qds) for 3 days with a view to discontinuing these medications if any blood sugars are ≤5mmol/L. *Note:* it is not unusual for insulin doses in excess of 100u per day to be discontinued in these patients once the liquid low calorie diet regime has begun.
- Referral to other clinical teams is made as appropriate e.g. cardiology, surgical.

Dietary strategy: meals and supplementation:

- Patient is commenced on low calorie milk based diet (see Appendix 3). Other food based options (a conventional calorie controlled diet) may be considered if the milk diet is not tolerated by individuals.
- Nutritional supplements are advised to ensure the liquid low calorie diet is nutritionally complete, which are:
    1. Multi-vitamin (Sona Balance, Sona Multiplus or Boots A-Z multivitamin & mineral) – once daily
    2. Omega 3,6,9 fatty acids (Omecor) – once daily
- In addition patients are recommended to drink a salty drink daily (e.g. Stock cube or ½ teaspoon salt in water) to meet sodium requirements
- Patients are also commenced on a bulking laxative (e.g. Fybogel) in an effort to prevent constipation. If patients are already on laxative therapy, this should be continued.

Programme inclusion criteria and specifications:

- Of note, all cases being considered for commencement are discussed by the Bariatric MDT.
- BMI >35 kg/m^2
- A maximum of three patients may be commenced on the milk diet at each bi-weekly clinic.

Exclusion criteria:

- Age <18 or >75 years
- Pregnancy or breastfeeding
- Significant renal disease
- Significant cardiac disease
- Recent MI or CVA
- Uncontrolled hypothyroidism
- Significant learning difficulties
- Significant psychiatric disorders
- Inability to commit to clinic visit schedule
- Inability to tolerate milk

Initiation of IMWP: procedures:

- Body weight, height, blood pressure, baseline ECG recorded.
- Full history and physical examination by medical team, CNS & Dietitian, including a comprehensive nutrition history.
- Programme protocol discussed with patient, including potential risks and benefits.
- Nutritional requirements calculated and a dietary regimen is devised for the patient by dietitian.
- Coping strategies and distraction techniques with respect to hunger are discussed with the patient prior to initiation. Written information is provided.

- Weight loss goals and time frames are discussed with the patient.
- Baseline blood tests taken.
- Smoking cessation advice and resources given, when appropriate.

Ongoing monitoring (weight loss phase – weeks 1 to 8):

- Patient on low-calorie milk-based diet only, as described below.
- Fortnightly visits to clinic, with body weight and blood pressure monitoring.
- Medication reviewed by medical team.
- Patient is reviewed by the Dietitian.
- The weight loss phase is used as an opportunity to provide education on various topics such as:
    - Record keeping
    - Physical activity
    - Environmental control
    - Food labelling
    - Calories in foods
    - Healthy eating
    - Weight loss maintenance
- Blood test taken, which include (but are not restricted to) urea & electrolytes, LFT, renal profile, FBC, TFT's, lipid profile, HbA1c.

Food reintroduction phase (weeks 8 to 16):

Weeks 1 & 2: Patients are asked to include a meal (dinner) consisting of a specific quantity of a protein food as well as a specific quantity of vegetables. This meal should be weighed using a digital food scales. The milk volume allocation should be reduced accordingly. The protein requirements of the patient should continue to be met by the diet. Monitoring should otherwise continue as in phase 1.

Weeks 3 & 4: Patients are asked to include 2 meals per day (lunch & dinner) consisting of 2 portions of protein foods, 4 portions of vegetables and 2 portions of carbohydrate foods. All foods should continue to be weighed. The milk volume is reduced substantially at this stage. Monitoring should otherwise continue as in phase 1.

Weeks 5 & 6: Patients are asked to include 3 meals per day (breakfast, lunch & dinner). Individual preferences should be taken into account. Again milk volume should be reduced as appropriate. Monitoring should otherwise continue as in phase 1.

Weeks 7 & 8: Patients can introduce some low calorie snacks into their diet. The full food reintroduction phase is now complete. Milk can continue to be included within healthy eating guidelines. At this stage a healthy eating regimen should be established in line with healthy eating guidelines. Monitoring should otherwise continue as in phase 1.

Weight maintenance phase (week 16-24):

- Patients continue to attend every 2 weeks.
- Monitoring continues as per phase 1 & 2.
- Issues such as relapse management, planning meals in advance, managing difficult meal timing and goal setting. Self-monitoring is vital at this stage and participants are encouraged to continue this practice on discharge.

Ongoing Support:

On completion of the IWMP, patients are followed up to ensure continued support and to aid with relapse management. Upon completion they are offered follow up appointments after 8 weeks, 16 weeks, 24weeks and afterwards if requested.

Premature discharge of participant from IWMP:

- Self-discharge
- Not tolerating milk volume
- Adverse effects as a results of programme
- Non-compliance with milk/laxatives/multivitamins
- Multiple lapses resulting in participants consuming excess calories
- Non-attendance at appointments
- On instruction from medical team

Low calorie milk-based diet outline:

Time	**Allowance**
8am	325ml semi-skimmed milk
10am	325ml semi-skimmed milk
12pm	325ml semi-skimmed milk
2pm	Either: 1 stock cube in a glass of water or 1 small sachet of salt in a glass of water
3pm	325ml semi-skimmed milk
6pm	325ml semi-skimmed milk
8pm	325ml semi-skimmed milk
10pm	325ml semi-skimmed milk

Supplements:

- Centrum Advance or Boots A-Z multivitamin (one a day)
- Omega 3,6,9 supplement.
- Fybogel 1-2sachets per day.

Notes:

- Unrestricted: Water, Tea, Coffee (no sugar; milk used from allowance above)
- Maximum 500mls “diet” minerals.

Summary:

Daily dietary intake	**Quantity**	**Electrolytes**	**Quantity**
Fluid volume	2275 ml	Sodium	53 mmol
Energy	1045 kcal	Potassium	87 mmol
Protein	75 g	Calcium	68 mmol
Fat	36 g		
Carbohydrate	113 g		

Nutritional intervention details:

Composition of the milk based low calorie diet (Luton & Dunstable hospital low calorie liquid diet)

	2272 ml (4 pints) Semi-skimmed milk	**SonaBalance or Boots A-Z multivitamin (1 dose)**	Total	**Irish RDA 1999 (18-64yr)**	
				Male	Female
Energy	1045 kcal	0 kcal	1045 kcal		
Protein	75g	0 g	75 g	0.75g/kg/day	
Fat	36g	0 g	36 g		
n-6 PUFA	Trace	0		2 % of dietary energy	
n-3 PUFA	Trace	0		0.5% of dietary energy	
Carbohydrate	113g	0 g	113g		
Sodium	53 mmol	0 mmol	53 mmol		
Potassium	87 mmol	0	87 mmol	79 mmol	79 mmol
Calcium	2726 mg	162 /200mg	mg	800 mg	800 mg
Magnesium	272 mg	100 /60mg	372/332 mg		
Phosphorus	2135 mg	109 /0mg	mg	550 mg	550 mg
Iron	1.36 mg	18 /14mg	mg	10 mg	14 mg
Zinc	9 mg	15 /10mg	24 /19mg	9.5 mg	7 mg
Vitamin D	22.7 ug	10/5 ug	32.7/27.7 ug	0-10 ug	0-10 ug
Vitamin K		25/75ug	25/75 ug		
Vitamin A		1050 /400ug	1050/400ug		
Vitamin E	68 mg	30/12 mg	98/80 mg		
Vitamin C	22.7 mg	60/80 mg	82.7/102.7mg	60 mg	60 mg
Vitamin B6	1.36 mg	2/1.4 mg	3.36/2.76 mg	15 ug / g protein	
Thiamine	0.9 mg	1.5 /1.1mg	2.4/2 mg	100 ug / MJ	
Riboflavin	3.86 mg	1.7/1.4 mg	5.6/5.26mg	1.6 mg	1.3 mg
Niacin	2.27 mg	20/16 mg	22.27/18.27 mg	1.6 mg/MJ	
Vitamin B12	9.08 ug	6/2.5 ug	11.58 ug	1.4 ug	1.4 ug
Folate	113 ug	400/200 ug	513/313 ug	300 ug	300 ug

RDA, recommended daily allowance;

PUFA, polyunsaturated fatty acids.

Low calorie dietary regimen following food re-introduction in phase two/ three:

Meal	**Food**	**Kcals**	**Protein g**
Breakfast	• 2 slices of brown / white Bread • 1 pat of Low Low • Tea / coffee (no sugar)	140 25	4 0
Mid-morning	• 1 medium piece of fruit	40	0
Lunch	• 1 scoop of mashed potato • 2 slices of meat / 1 breast of chicken (no breadcrumbs or batter) / 1 fillet of fish (~ 3oz) • 4 tablespoons veg of the Day • 200ml low fat milk • 1 diet yoghurt	70 130 – 180 40 90 60	2 21–30 1 7 7
Mid-afternoon	• 1 medium piece of fruit	40	0
Tea	• 2 slices of white / brown Bread • 1 pat Low-Low • 1 small tin of tuna / 2 oz hard cheese / 2 slices of ham / 1 chicken breast / 2 boiled eggs • Heaped plate of lettuce / tomato / onion / sweetcorn / beetroot / mixed bean salad NO coleslaw/potato salad/mayonnaise/ dressing • Tea / coffee (no sugar)	140 25 180 – 240 40	4 0 14–21 1 – 7
Before bed	• 1 diet yoghurt	60	7
Total		1134	75

Patient Information Leaflet

The **GERONIMO** Study

Genetic **E**ffects on the **R**esponse to an **O**utpatient Intensive **N**utritional **I**ntervention in **M**edically Complicated **O**besity

You have been invited to take part in this study because you were previously treated with the 'MILK DIET' at the Bariatric Medicine Clinic in University Hospital Galway.

While attending this clinic, information about your medical history, medications, blood test results and measurements such as weight and height were routinely recorded. These records are confidentially and securely stored within Galway University Hospitals. As part of the GERONIMO study, we would like to analyse this information to assess the effectiveness of the care we provide to patients. Our aim is to inform better care for individual patients in the future. By using data from individual patients in this way, we can conduct valuable and important medical research that will ultimately lead to more effective and efficient patient care.

We would also like to investigate if genes influence weight loss response to the 'MILK DIET'. We are asking all patients who completed the 24 week programme to provide a blood sample from which we will obtain DNA, in order to better understand the genetic influences on weight loss. We would like to measure leptin and adiponectin, the hormones produced by adipocytes/ fat cells as they are involved in inflammation and insulin resistance in some patients. We would also like to measure blood ketone levels as a standard blood test in all our patients during the milk programme. No data that we use will ever be personally identifiable, nor will participating or declining this opportunity influence the nature of the care that you receive in any way, now or in the future. If you agree to take part in the study, you will be asked to come to the Clinical Research Facility in University Hospital Galway for a 10 minute appointment. **You will need to fast for 12 hours prior to this appointment.**

When you first arrive at the hospital, you will meet one of our research fellows and will be given the opportunity to ask any questions you may have about the study. If you are happy to proceed you will be asked to sign a consent form. You will be provided with a copy of this consent form for your own personal records.

We will measure your height and weight and will estimate your total body fat composition. We will also take measurements of your waist, thigh and hip. we would also like to measure your blood pressure.

Please note that the doctors involved in this study are covered by standard medical malpractice insurance. Nothing in this document or in the consent form for the study restricts or curtails your rights in any way.

Thank you for reading this information leaflet. Please note that it is intended to complement the other information you have received, and we would be delighted to discuss any queries you might have at any time.

Genetic **E**ffects on the **R**esponse to an **O**utpatient Intensive **N**utritional **I**ntervention in **M**edically Complicated **O**besity: The **GERONIMO** Study.

While attending the bariatric services in the hospital, information about your medical history, medications, blood test results (this will include blood ketone levels) and measurements such as weight and height are routinely recorded. These records are confidentially and securely stored, indefinitely, within Galway University Hospitals. As part of a research study, we would like to analyse this information to assess the effectiveness of the care that we provide to patients. Our aim is to inform better care for individual patients in the future. In particular, we would like to learn more about how patients respond to the "milk diet". By using data from individual patients in this way, we can conduct valuable and important medical research that will ultimately lead to more effective and efficient patient care. Secondly, we would like to see whether genes influence how well individuals respond to the milk diet. We are asking all patients who completed the programme to provide a blood sample, from which we will obtain DNA, in order to better understand the genetic influences on weight loss. We would also like to measure leptin and adiponectin levels, these are hormones produced by the adipocytes/fat cells and are involved in the process of inflammation in our body. No data that we use will ever be personally identifiable, nor will participating or declining this opportunity influence the nature of the care that you receive in any way, now or in the future.

Please initial each box

1. I confirm that I have read and understood the information detailed above, and have been given the opportunity to ask questions. ☐

2. I agree to have information relating to my medical history, medications, blood tests and body size measurements recorded during my visit and for this information to be confidentially and securely stored, indefinitely, within Galway University Hospitals. ☐

3. I consent to the results of any analysis of these data being published for scientific purposes, either in print media or electronically online, now or in the future. ☐

4. I understand that the data recorded during my visit will be anonymised whenever they are used for research purposes. ☐

5. I consent for a sample of blood to be analysed for genes that have been identified now or will be in the future that are associated with obesity and diabetes. I understand that the nature of this research means that genetic information obtained from these tests will not be made available to me personally, or to my doctor or any third party now or at any time in the future. ☐

6. I understand that I am free to withdraw consent for my data being used for research purposes at any time, without giving any reason and without my medical care or legal rights being affected in any way, now or in the future. ☐

----------	----------	----------Name
of Patient (BLOCK CAPITALS)	Date	Signature
----------	----------	----------Name
of Researcher	Date	Signature

ALT

cobas®

Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation Order information

REF	CONTENT		Analyzer(s) on which cobas c pack(s) can be used
05850797 190	Alanine Aminotransferase acc. to IFCC (1100 tests)	System-ID 05 7501 0	Roche/Hitachi cobas c 701/702
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
12149443 160	Precipath U plus (10 x 3 mL, for USA)	Code 301	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
05172152 190	Diluent NaCl 9 % (119 mL)	System-ID 08 6869 3	

English

System information

ALTL: ACN 8685

Intended use

In vitro test for the quantitative determination of alanine aminotransferase (ALT) in human serum and plasma on Roche/Hitachi cobas c systems.

Summary[1,2]

The enzyme alanine aminotransferase (ALT) has been widely reported as present in a variety of tissues. The major source of ALT is the liver, which has led to the measurement of ALT activity for the diagnosis of hepatic diseases. Elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, carcinoma of the liver, and chronic alcohol abuse. ALT is only slightly elevated in patients who have an uncomplicated myocardial infarction.

Although both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver-specific enzyme. Moreover, elevations of ALT activity persist longer than elevations of AST activity.

In patients with vitamin B_6 deficiency, serum aminotransferase activity may be decreased. The apparent reduction in aminotransferase activity may be related to decreased pyridoxal phosphate, the prosthetic group for aminotransferases, resulting in an increase in the ratio of apoenzyme to holoenzyme.

Test principle

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.[3,4]

ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD^+.

$$\text{L-Alanine} + \text{2-oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{L-glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-lactate} + NAD^+$$

The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.

Reagents - working solutions

R1 TRIS buffer: 224 mmol/L, pH 7.3 (37 °C); L-alanine: 1120 mmol/L; albumin (bovine): 0.25 %; LDH (microorganisms): ≥ 45 µkat/L; stabilizers; preservative

R3 2-Oxoglutarate: 94 mmol/L; NADH: ≥ 1.7 mmol/L; additives; preservative

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines.

Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Reagent handling

Ready for use

Storage and stability

ALT

Shelf life at 2-8 °C:	See expiration date on cobas c pack label.
On-board in use and refrigerated on the analyzer:	4 weeks
On-board on the Reagent Manager:	24 hours

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on cobas c pack label.
On-board in use and refrigerated on the analyzer:	4 weeks
On-board on the Reagent Manager:	24 hours

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

Plasma: Li-heparin and K_2-EDTA plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Separate the serum or plasma from the clot or cells promptly.

Centrifuge samples containing precipitates before performing the assay. See the limitations and interferences section for details about possible sample interferences.

ALT

Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Stability:	3 days at 15-25 °C[5]
	7 days at 2-8 °C[5]
	> 7 days at (-60)-(-80) °C

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- See “Order information” section
- General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 701/702 test definition

Assay type	Rate A		
Reaction time / Assay points	10 / 24-38		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	65 µL	36 µL	
R3	19 µL	22 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	10 µL	–	–
Decreased	10 µL	15 µL	135 µL
Increased	20 µL	–	–

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration
	- after reagent lot change
	- as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the original IFCC formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.[6]

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi cobas c systems automatically calculate the analyte activity of each sample.

Conversion factor: U/L x 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at an ALT activity of 30 U/L (0.5 µkat/L).

Icterus:[7] No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[7] No significant interference up to an H index of 90 (approximate hemoglobin concentration: 56 µmol/L or 90 mg/dL).

Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid):[7] No significant interference up to an L index of 150. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Lipemic samples may cause > Abs flagging. Choose diluted sample treatment for automatic rerun.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[8,9]

Exception: Calcium dobesilate can cause artificially low ALT results at therapeutic concentrations.

Cyanokit (Hydroxocobalamin) may cause interference with results.

Physiological plasma concentrations of Sulfasalazine or Sulfapyridine may lead to false results.

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[10]

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi cobas c systems. All special wash programming necessary for avoiding carry-over is available via the cobas link, manual input is required in certain cases. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SmpCln1+2/SCCS Method Sheet and for further instructions refer to the operator’s manual.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

5-700 U/L (0.08-11.7 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Lower detection limit of the test

5 U/L (0.08 µkat/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Values below the lower detection limit (< 5 U/L) will not be flagged by the instrument.

ALT

cobas®

Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation

Expected values[11]

Acc. to the optimized standard method (comparable to the IFCC method without pyridoxal phosphate activation[12]):

Males	up to 41 U/L	(up to 0.68 µkat/L)
Females	up to 33 U/L	(up to 0.55 µkat/L)

Calculated values: A factor of 1.85 is used for the conversion from 25 °C to 37 °C.[13]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol. Repeatability (n = 21), intermediate precision (3 aliquots per run, 1 run per day, 20 days). The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	%
Precinorm U	46.3 (0.773)	0.8 (0.013)	1.6
Precipath U	123 (2.05)	1 (0.02)	0.5
Human serum A	21.5 (0.359)	0.7 (0.012)	3.1
Human serum B	80.3 (1.34)	0.4 (0.01)	0.5
Human serum C	546 (9.12)	3 (0.05)	0.6

Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	%
Precinorm U	39.3 (0.66)	0.6 (0.01)	1.4
Precipath U	120 (2.00)	1 (0.02)	1.0
Human serum 3	24.0 (0.40)	0.6 (0.01)	2.6
Human serum 4	98.1 (1.64)	3.2 (0.05)	3.3

Results for intermediate precision were obtained on the master system cobas c 501 analyzer.

Method comparison

ALT values for human serum and plasma samples obtained on a Roche/Hitachi cobas c 701 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi cobas c 501 analyzer (x).

Sample size (n) = 69

Passing/Bablok[14]	Linear regression
y = 0.976x + 1.61 U/L	y = 0.971x + 2.82 U/L
τ = 0.985	r = 0.999

The sample activities were between 5.60 and 637 U/L (0.094 and 10.6 µkat/L).

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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